The 8th Congress of Toxicology in Developing Countries (8CTDC)

September 10-13, 2012- Bangkok, Thailand

"Sharing Toxicological Knowledge for Healthy Life & Environment"

PROGRAME AND ABSTRACTS

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WELCOME MESSAGE FROM CONGRESS PRESIDENT

Welcome all participants, speakers, IUTOX Executive members and guests,

It is my pleasure to greet all of you to the 8th Congress of Toxicology in Developing Countries (8CTDC) in Bangkok held at Centara Grand Hotel at Central Plaza Ladprao. This congress is hosted by Thai Society of Toxicology under the auspices of International Union of Toxicology (IUTOX) headed by Professor Dr. Daniel Acosta, Jr., the President

The congress is on 10-13 September 2012 and consists of one keynote lecture, 3 plenary lectures, 11 symposia. One workshop as continuing education is also held one day before the main started, entitle "**WHO Human Health Risk Assessment Toolkit for Chemical Hazards**". The congress invites more than 40 speakers internationally to present their update on the subjects. The honorable keynote lecture is presented by Professor Dr. HRH Princess Chulabhorn on the title of **"Environmental Carcinogens: Exposure and Impact on Children's Health".** This is the very important area that contributes to the progress in Toxicology. The three plenary lectures will add to the update on the fields of genetic polymorphism and toxicology, nuclear toxicology, natural animal toxin and its applications. The symposia are ranging from molecular, clinical, environmental, natural products, herbal medicinal, occupational, food, regulatory, and testing, to assessment toxicology. Additionally, there are 28 oral and more than 120 poster presentations on new finding of research are also presented.

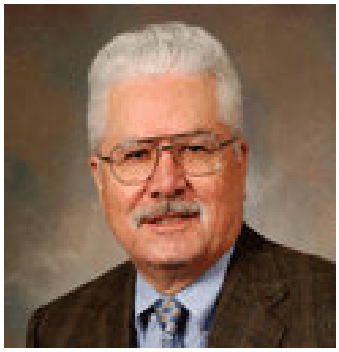
I am sure that this congress will add more knowledge and information to your present one. The update information and research outcome presented in lectures, symposia, and oral and paper presentations will benefit to you and your work. It is also a good opportunity for you all to exchange of information, idea and work among participants around the world. It is also a good chance to meet old friends and make new ones for future collaboration.

During your stay in Bangkok, please find sometimes to look around the area which is rich in culture and places of interest. I wish all participants a good stay and enjoy both the science of toxicology in the congress and the sightseeing in Thailand, the land of smile.

S. Srianujata

Associate Professor Dr. Songsak Srianujata
President, Thai Society of Toxicology
Chairman of the Organizing Committee for 8CTDC
August, 2012

WELCOME MESSAGE FROM IUTOX



On behalf of IUTOX and our 61 Member Societies that span the globe, welcome to the 8th Congress of Toxicology in Developing Countries (CTDC8). Under the strong leadership of Dr. Songsak Srianujata, the Thai Society of Toxicology has planned a superb scientific program around the timely theme, "Sharing Toxicological Knowledge for a Healthy Life and Environment." The Congress has attracted prominent scientists from around the world to lecture on diverse topics covering many aspects of toxicology.

IUTOX is very grateful to Professor Dr. HRH Princess Chulabhorn Mahidol, a renowned scientist and founder of the Chulabhorn Research Institute, for her commitment and leadership in the field of toxicology. We are especially grateful for her participation in the Opening Ceremony and for serving as the keynote lecturer.

We also extend a hearty welcome and thank you to the World Health Organization for providing the Congress with excellent programming including the Continuing Education Program on risk assessment tools for chemical hazards, and a second session, which addresses the environmental burden of disease in developing countries. WHO consistently supports IUTOX and the thousands of scientists we represent with training and programs in developing countries, which are critically important to helping us meet our mission.

When the IUTOX Member Societies selected Bangkok as the host city of the CTDC8 meeting, they may have imagined a venue marked by beauty, grace and elegance. Surely, those who are lucky enough to join us this week will not be disappointed. Bangkok dazzles visitors with ancient treasures nestled alongside a modern transportation system and towering skyscrapers. This *City of Angels* and the gracious people of Bangkok have provided a beautiful, welcoming venue for an unforgettable congress.

We wish you an exciting week marked by the pursuit of outstanding science and knowledge and the delights of new friendships amid a truly magnificent city.

Daniel Acosta, Jr.

Daniel Acosta, Jr., Ph.D., ATS
President, IUTOX

ACKNOWLEDGEMENTS

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MONDAY, 10th SEPTEMBER 2012		
		Room
09.00-15.00	**Continuing Education Program:** WHO Human Health Risk Assessment: Toolkit for Chemical Hazards	Rangsit 2-3
09.30-15.00	Registration	Pre-function Vibhavadee Ballroom C
16.00-16.30	Opening Ceremony	Vibhavadee Ballroom B
16.30-17.30	**Keynote Lecture**: **Environmental Carcinogens: Exposure and Impacts on Children's Health** *Professor Dr. HRH Princess Chulabhorn Mahidol (Thailand)*	Vibhavadee Ballroom B
18.00-22.00	Welcome Reception	Vibhavadee Ballroom A

TUESDAY, 11th SEPTEMBER 2012		
		Room
08.00-16.00	**Registration**	
08.45-09.30	**Plenary Lecture I:** Gene-environment Interaction in Toxicity *Herman Autrup (Denmark)* **Chairperson:** Songsak Srianujata (Thailand)	Vibhavadee Ballroom B
09.30-10.00	**Coffee/Tea Break**	Pre-function area
Room	**Vibhavadee Ballroom B**	**Vibhavadee Ballroom A**
10.00-12.00	**Symposium I:** ***Susceptible Populations Including Genetic Polymorphism*** **Chairpersons:** Elaine Faustman (USA) Wongwiwat Tassaneeyakul (Thailand)	**Symposium II:** ***Clinical Toxicology*** **Chairpersons:** Chen-Chang Yang (Taiwan) Winai Wananukul (Thailand)

	1.1 Gene time environment: Considerations in identifying at risk families in agricultural communities *Elaine Faustman (USA)* 1.2 Drug/xenobiotic metabolizing enzyme polymorphisms in the clinical outcome in lung cancer *Mumtaz Iscan (Turkey)* 1.3 Role of drug-metabolizing enzymes in drug-induced toxicity: Influence of microRNA and immune-related factors *Tsuyoshi Yokoi (Japan)* 1.4 Genetic polymorphisms and breast cancer susceptibility in Thai women *Suleeporn Sangrajrang (Thailand)*	2.1 The role of clinical toxicologist in the management of acute and/or chronic poisoning outbreaks: Experience of the Taiwan National Poison Control Center *Chen-Chang Yang (Taiwan)* 2.2 Clinical toxicology in Sri Lanka: Translating research into practice *Andrew Dawson (Australia)* 2.3 Poisoning issues in Vietnam *Pham Due (Vietnam)*
12.00-13.00	**Lunch**	Ladprao Suite
13.00-14.00	**Poster Discussion**	Pre-function area
Room	**Vibhavadee Ballroom B**	**Vibhavadee Ballroom A**
14.00-16.00	**Symposium III:** ***New Approaches to Toxicity Testing and Risk Assessment*** **Chairpersons:** Emanuella Corsini (Italy) Chaniphun Butryee (Thailand) 3.1 Frontier of 3D-culture technologies for extrapolating toxicity *in vivo* *Toshiaki Takezawa (Japan)* 3.2 RISK 21 *Samuel M. Cohen (USA)* 3.3 A novel biomarker to detect endocrine disrupting chemicals for risk assessment: Do they have an estrogenicity or tumorigenicity in cellular models? *Kyung-Chul Choi (South Korea)* 3.4 New approaches in immunotoxicology *Emanuella Corsini (Italy)*	**Symposium IV:** ***New Era of Herbal Medicines and Dietary Supplements*** **Chairpersons:** Young-Joon Surh (South Korea) Porntipa Picha (Thailand) 4.1 Kefiran reduces atherosclerosis in rabbits fed a high cholesterol diet *Itsuko Ishii (Japan)* 4.2 Effects of lignans from sesame seed on genomic and non-genomic estrogen signalling *Apinya Thiantanawat (Thailand)* 4.3 Health beneficial effects of phytopharmaceuticals and phytonutrients: Xenohormetic perspectives *Young-Joon Surh (South Korea)* 4.4 Safety re-evaluation of Chinese traditional medicine in injection formulation *Quanjun Wang (China)*
16.00-16.30	**Coffee/Tea Break**	

Room	Vibhavadee Ballroom B	Vibhavadee Ballroom A
16.30-17.30	**Free Paper I** **Chairperson:** Panida Navasumrit ❖ Immunomodulatory effects of ethanolic extract of *Thyphonium flagelliforme* (Lodd.) Blume on cyclophosphamide−treated rats *Arief Nurrochmad (Indonesia)* ❖ Histopathological and biochemical changes induced in rabbits by prolonged oral cyanide intoxication *Muhammad Avais (Pakistan)* ❖ Development of Ah (dioxin) receptor-based approaches for the study of toxicology and health effects of dioxins and related chemicals *Bin Zhao (China)* ❖ A comparison study of single and combined cytotoxic effects of fumonisin B1, aflatoxin B1 and ochratoxin A on human mononuclear blood cells using methyl tetrazolium assay, comet assay and flow cytometry *Mulunda Mwanza (South Africa)* ❖ Risk evaluation of exposure to low concentration of carcinogenic mycotoxins – molecular evidence of synergystic effects *Annie Pfohl-Leszkowicz (France)*	**Free Paper II** **Chairperson:** Sirinmas Katchamart ❖ Risk based approaches to ensure safety of cosmetics: prevention of skin sensitisation *Nicola J Gilmour (UK)* ❖ Taurine an early biomarker of drug-induced liver necrosis *Mohammadreza Sattari (Iran)* ❖ Understanding the mechanism of lapatinib-induced hepatotoxicity: An *in vitro-in vivo* investigation *Han Kiat Ho (Singapore)* ❖ Study of incidence of amphotericin B side effects and nephrotoxicity risk factors in patients under chemotherapy *Simin O. Mashayekhi (Iran)*

WEDNESDAY, 12th SEPTEMBER 2012		
		Room
08.45-09.30	**Plenary Lectures II:** Vibhavadee Ballroom B **Radiation toxicology - Differences and Similarities Between Radiation and Chemicals** *Jun Kanno (Japan)* **Chairperson:** Malyn Chulasiri (Thailand)	
09.30-10.00	**Coffee/Tea Break** Pre-function area	
Room	**Vibhavadee Ballroom B**	**Vibhavadee Ballroom A**
10.00-12.00	**Symposium V:** ***Environmental Burden of Disease in Developing Countries: From Evidence to Action*** **Chairpersons:** Herman Autrup (Denmark) Songsak Srianujata (Thailand) 5.1 Burden of disease attributable to chemicals and air pollution: Overview of the evidence *Kersten Gutschmidt (Switzerland)* 5.2 Using evidence to prevent deaths from pesticide poisoning in rural Asia *Michael Eddleston (UK)* 5.3 Reducing the burden of disease from metals through regulation and policy measures: Successes and possibilities *Joanna Tempowski (Switzerland)* 5.4 Health impact from hazardous waste sites in India, Indonesia, and the Philippines *Kevin Chatham-Stephens (USA)*	**Symposium VI:** ***Occupational Toxicology*** **Chairpersons:** Salmaan H. Inayat-Hussain (Malaysia) Suleeporn Sangrajang (Thailand) 6.1 Occupational exposure to mercury in an oil and gas industry: Confounding effects of seafood intake in biomonitoring of blood *Salmaan H Inayat-Hussain (Malaysia)* 6.2 Occupational and environmental bladder cancer risk and genetic susceptibility *Klaus Golka (Germany)* 6.3 PAH and crystalline silica dust exposure-related hazards in occupational environments in turkey *Sema Burgaz (Turkey)* 6.4 Rational use of animal toxicology data in setting up occupational exposure of chemicals to humans *K.S. Rao (India)*
12.00-13.10	**Luncheon Symposia** Vibhavadee Ballroom B **Session Title: Food Safety and Risk Communication** • Risk management and risk communication of food *Hideaki Karaki, Ex-President of Japanese Society of Toxicology* *Expert Member of Food Safety Commission, Cabinet Office, Government of Japan* • Misconception of food ingredient safety: Case of Glutamate *John D. Fernstrom, Professor of Psychiatry, University of Pittsburgh School of Medicine* **Chairperson:** Songsak Srianujata President of Thai Society of Toxicology	
13.10-14.00	**Poster Discussion** Pre-function area	

Room	Vibhavadee Ballroom B	Vibhavadee Ballroom A
14.00-16.00	**Symposium VII:** ***Toxicological Aspects of Nanoparticles*** **Chairpersons:** Ali Esat Karakaya (Turkey) Sirirurg Songsivilai (Thailand) 7.1 Roadmap for nonosafety - a mission impossible? *Kai Savolainen (Finland)* 7.2 Size dependent translocation pattern, chemical transformation and toxicity of ferric oxide nanoparticles in the central nervous system *Wang Bing (China)* 7.3 Toxicity testing and intracellular uptake of gold nanoparticles *Mary Gulumain (South Africa)* 7.4 National nanosafety and ethics strategic plan: Thailand's perspective *Sirirurg Songsivilai (Thailand)*	**Symposium VIII:** ***Regulatory Toxicology Relating to Industry*** **Chairpersons:** Tetsuo Satoh (Japan) Pensri Watchalayann (Thailand) 8.1 Progress in the European Chemicals legislation REACH and Impact on Toxicology *Heidi Foth (Germany)* 8.2 Pesticide regulation *Lewis Smith (UK)* 8.3 Regulatory science for the safety evaluation in non-clinical research and development *Ikuo Horii (Japan)* 8.4 Chemical risk assessment and management in China: History and progresses *Lijie Fu (China)*
16.00-16.30	**Coffee/Tea Break**	Pre-function area
Room	**Vibhavadee Ballroom B**	**Vibhavadee Ballroom A**
16.30-17.30	***Free Paper III*** **Chairperson:** Jintana Sirivarasai ❖ Hormonal disturbances caused by benzene in rat *S.V.S. Rana (India)* ❖ Increased blood heme oxygenase-1 levels in high silica exposure non-silicosis stone mill workers *Kowit Nambunmee (Thailand)* ❖ Heavy metals in pig production : A concern for public health *S. Durosoy (France)* ❖ Quantification of pesticide use in Egypt *Salah A. Soliman (Egypt)*	***Free Paper IV*** **Chairperson:** Sarisak Soontornchai ❖ Are the new Chinese chemicals regulations catching up with REACH? *Qian Ding (Sweden)* ❖ Carboxydextran-coated superparamagnetic iron oxide nanoparticles affect the long-term fate of kupffer cells and macrophages *Tatiana Syrovets (Germany)* ❖ Involvement of ROS generation in silver nanoparticle-induced A-549 cell toxicity *Rawiwan Maniratanachote (Thailand)* ❖ Genotoxic effects *in vitro* of exposure to nanoparticles with different physico-chemical properties *Konrad Rydzyński (Poland)* ❖ Innovations towards a non toxic environment and holistic health in Developing Countries and beyond *Palarp Sinhaseni (Thailand)*
18.00-22.00	**Gala Dinner**	Suan Bua

THURSDAY, 13th SEPTEMBER 2012		
		Room
08.45-09.30	**Plenary Lecture III:** **Clinical Physiology of Animal Toxins : Kidney Model** *Visith Sitprija (Thailand)* **Chairperson:** Sumol Pavittranon (Thailand)	Vibhavadee Ballroom B
09.30-10.00	**Coffee/Tea Break**	Pre-function area
Room	**Vibhavadee Ballroom B**	**Vibhavadee Ballroom A**
10.00-12.00	**Symposium IX:** ***Molecular Carcinogenesis and Chemoprevention*** **Chairpersons:** Young Nam Cha (South Korea) Rodjana Chunhabundit (Thailand) 9.1 Metabolic dysregulation and its clinical implication in ovarian cancer *Yong Sang Song (South Korea)* 9.2 Natural compounds as modulators of intracellular signalling pathways and oxidative stress in mammalian cells and *Caenorhabditis elegans* *Wim Waetjen (Germany)* 9.3 Selected West African phytochemicals and chemoprevention *Olatunde Farombi (Nigeria)*	**Symposium X:** ***Foodborne Toxins in Developing Countries*** **Chairpersons:** Kevin Chipman (UK) Anong Bintvihok (Thailand) 10.1 Saxitoxins in fresh waters *Ernani Pinto (Brazil)* 10.2 Recent review of marine toxin poisoning in Taiwan *Deng-Fwu Hwang (Taiwan)* 10.3 Food-derived furan exposure, mechanisms of carcinogenic action and risk assessment *Kevin Chipman (UK)*
12.00-13.00	**Lunch**	Ladprao Suite
Room		Vibhavadee Ballroom B
13.00-14.15	**Plenary Symposia:** ***Herbal Product Safety*** **Chairpersons:** Jou-Fang Deng (Taiwan) Summon Chomchai (Thailand) • Medicinal uses of aconite roots-rationalising research and risk communication strategies *Thomas Y.K. Chan (Hong Kong, China)* • The spectrum of herbal poisoning *Jou-Fang Deng (Taiwan)* • Traditional medicine related poisoning *Winai Wananukul (Thailand)*	
14.15-14.30	**Poster Awards/ Closing Ceremony**	

ABSTRACTS

ENVIRONMENTAL CARCINOGENS: EXPOSURE AND IMPACTS ON CHILDREN'S HEALTH

HRH Princess Chulabhorn Mahidol, Ph.D.

Chulabhorn Research Institute, Bangkok 10210, Thailand

Environmental factors play a major role in determining the health and well being of children who comprise over one third of the world's population. Arsenic and carcinogenic compounds in air pollution are examples to illustrate how exposure to these compounds can potentially impact children's health. Prenatal arsenic exposure in a human population resulted in alarming gene expression changes in the newborns. Class prediction algorithms identified gene expression signatures that predict arsenic exposure in a test population with about 80% accuracy. A highly predictive potential biomarker gene set composed of just 11 genes was identified. These genes are promising as genetic biomarkers for prenatal arsenic exposure. There is a robust prenatal response that correlates with arsenic-exposure levels that could modulate numerous biological pathways including apoptosis, cell signaling, inflammatory response, and other stress responses, and ultimately affect health status. The health impact of exposure to environmental carcinogens in air pollution during childhood was also examined. Personal monitoring of exposure and urinary metabolite excretion showed that city school children were exposed to benzene, 1,3-butadiene and PAHs at levels significantly higher than rural children, which was approximately 2-fold for benzene, 4-fold for 1,3-butadiene and 4-fold for PAHs. The early biological effects from exposure to carcinogens were assessed from DNA damage measured as 8-OHdG and DNA strand breaks and DNA repair capacity. 8-OHdG in leukocyte DNA which was 2.5 fold higher in the city school children compared with the rural school children was statistically significantly correlated with benzene exposure level. The levels of DNA strand breaks in peripheral blood samples from the city children were 1.5 fold higher than those in the rural children. Chromosome damage measured by the challenge assay were 1.7 fold higher in city children, indicating a reduction in DNA repair capacity in these children. Taken together, significantly higher levels of DNA damage believed to be the first step in development of cancer, coupled with a decreased DNA repair capacity, indicate that these children are at a higher risk for developing cancer later in life.

GENE-ENVIRONMENT INTERACTION IN TOXICITY

Herman Autrup

Institute for Public Health, University of Aarhus, Aarhus, Denmark

Large variation in response to drug and environmental toxicant has been reported, and it has been determined that gene-environment interaction plays an important role in the etiology of many chronic diseases, i.e. cancer. Large inter-individual variation in the activity of enzymes involved in the uptake and metabolism of the toxicant and the repair of the damage induced by the chemical has been has been reported. The genetic basis for this variation has in part been identified. In addition to this variation, environmental factors, including nutrition could influence the expression of the genes, i.e. phenotype. Thus influencing the dose of the bioactive molecule. Genetic variation in specific genes can indirectly influence risk as they may alter an individual's behavior and thus the exposure, e.g. smoking, alcohol consumption. Genetic polymorphisms have been detected in many xenobiotic metabolizing enzymes, e.g., members of the CYP450 and GST families resulting in different biological effects like increased expression, reduced or no activity and change in substrate specificity. These polymorphisms alter the formation of the biological active metabolite and thus the disease risk. A major focus of these first generation studies has been on the effect on biomarker levels, e.g., DNA adducts, and the development of cancer. As several gene products are involved in the metabolism of a chemical, the overall risk will be depending on the haplotype (multiple polymorphisms in the same gene) and on the effect of multiple genes as shown in case of DNA adducts and cancer risk. This effect is not only due to the genetic polymorphism but also the level of exposure. In chemical risk assessment a default assessment factor of 10 is normally used to account for inter-individual variation. Knowledge on the genetic variation may result in a science based assessment factor in order to protect the most susceptible individual.

RADIATION TOXICOLOGY –DIFFERENCES AND SIMILARITIES BETWEEN RADIATION AND CHEMICALS

Jun Kanno

Division of Cellular & Molecular Toxicology, Biological Safety Research Center, National Institute of Health Sciences, Tokyo, Japan

The approach of risk assessment and risk management is good for chemicals including genotoxic carcinogens found in the human environment including food. And radiation is also a genotoxic carcinogen basically shares common mechanisms with genotoxic carcinogens, i.e. caloric restriction attenuates the carcinogenic potential of radiation in experimental animal, and mutagenic potential of ethyl nitrosourea, a chemical carcinogen, is attenuated by pretreatment of low-dose radiation (so-called “tickle” dose). Therefore, it is rational to consider that the same risk assessment/management approach can be applied for the regulation of radioactive fallouts and contaminated foods. Unfortunately, such science once called Radiation Toxicology which handles chemical and radiation equally has been unpopular for long. Epidemiological data are very much limited to A-bomb survivors, whose radiation exposures were completed within a second. Biological effects of the continuous low dose exposure of external and internal radiation with and without the co-exposure of certain chemicals are not sufficiently understood in the context of radiation toxicology. Current government adopted the ICRP recommendation for management of radioactive and radiation until and after the incidence. However, the “Mund therapie” by a certain radiation experts given to the exposed population was also announced to the non-exposed people; “There is no scientific evidence that the cancer are clearly induced below the level of 100mSv, and hence, no need to fear”. “Stress will be more harmful, so do not seriously consider a small amount of radiation”. As an inevitable consequence of these messages, non-exposed people became anxious and skeptic on whatever the experts and the government say. The fundamental deficiency was the distinction of risk assessment or risk management; during assessment, different experts can present various ideas and opinion, whereas management, a selection is made for real action(s). And, for the latter process, an arrangement of the round-table meeting of all stakeholders is essential.

CLINICAL PHYSIOLOGY OF ANIMAL TOXINS : KIDNEY MODEL

Visith Sitprija

Queen Saovabha Memorial Institute Bangkok, Thailand

Animal toxins, through effects of their composition which includes enzymes, peptides, proteins and chemicals, can generate significant pathophysiological changes in the host. Hemodynamic changes and inflammation, induced by cytokines, vasoactive mediators and ion channel modulation, and their interaction can cause cellular injury. Some toxins can also exert direct tissue injury. Except anaphylaxis, immunologic reaction plays a minor role. The kidney, as a highly vascularized organ, is often the target of toxins and serves as an execellent model for venom study. All renal structures can be affected, and at the clinical level renal failure is common. Toxins can also modulate ion channels of epithelial cells. Yet, data are few in the literature. Fluid and electrolyte changes due to animal toxins are most interesting for exercise in physiology and deserve more attention.

MEDICINAL USES OF ACONITE ROOTS – RATIONALISING RESEARCH AND RISK COMMUNICATION STRATEGIES

Thomas Y.K. Chan[1,2]

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In traditional medicine, aconite roots (roots and root tubers of the *Aconitum* species) are used as medicinal plants only after adequate processing to markedly reduce their toxic alkaloid content. Processed aconite roots should only be prescribed and dispensed by a registered herbalist. The recommended dose should not be exceeded, i.e. 1.5-3.0 g for processed *Radix Aconiti* and processed *Radix Aconiti Kusnezoffii* and 3-15 g for processed *Radix Aconiti Lateralis*. Processed aconite roots should be decocted for ≥1.5-2 h before boiling with other herbs. Soaking and boiling during post-harvest processing and decoction preparation hydrolyse the *Aconitum* alkaloids (aconitine, mesaconitine, hypaconitine, yunaconitine, crassicauline A, jesaconitine, etc.) into less toxic and non-toxic derivatives. As these *Aconitum* alkaloids are potent cardiotoxins and neurotoxins with a narrow therapeutic index and herb-induced aconite poisoning can be complicated by serious cardiotoxicity leading to refractory ventricular tachyarrhythmias and asystole, extra caution is required in handling aconite roots (from quality control in post-harvest processing, trading, prescribing, dispensing to decoction preparation and consumption). To minimise the risks and maximise the potential benefits of aconite root therapy, there is the obvious need for a regulatory standard to ensure product safety/quality and good practices in the production, prescription, dispensing and administration of these herbs. A priority for research is to identify the avoidable factors and define the risks associated with aconite roots and unsatisfactory practices. There should be publicity measures to promote awareness of the toxicity of aconite roots and to emphasise the importance of appropriate dosing and compliance with instructions on decoction and consumption of these herbs. The public should also realise the high toxicity of aconite tincture, which is often prepared from raw aconite roots. Because of the presence of *Aconitum* alkaloids in very high concentrations, aconite tincture should never be taken by mouth. Effective communication strategies should be established to reach all target audiences.

THE SPECTRUM OF HERBAL POISONING

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Many of the natural toxins originated from animals, plants as well as microorganisms are well known of having the potential to produce prominent but unwanted health effects. Due to the increased morbidity and mortality, the poisonings associated with the use of herbs and/or traditional medicines has raised an universal attention in past few years. In daily practice, herbs and/or traditional medicines has been widely used for either therapeutic or tonic purpose. It was found of having been dispensed by health professionals, quacks and other non-medical professionals such as witch doctors. It may contain minerals or any part of plants and/or animals. Upon exposure, the untoward clinical effects may vary from mild to severe toxicity and even life-threatening. In clinical setting, the difficulty in handling the poisonings associated with the use of herbs and/or traditional medicines can be categorized in the aspects of: (1) identification of the proprietary substances and active ingredients; (2) characterizing the kinetic pattern and toxicological effects; (3) the potential interaction between the herbs and modern medicines taken by the patients; (4) uncertainty of the diagnosis and treatment. Since the content and pattern of the use of herbs and/or traditional medicines does vary with the ethic culture and geography, a monitoring program designed for international use shall be useful in creating a systemic international data bank of herbs and/or traditional medicines with potential toxicity. In the meanwhile, further researches such as: (1) to facilitate the upgrading of analytic capability of identifying the active ingredients and characterizing its kinetics; (2) to speed up the procedures of evaluating the pharmacological and toxicological impacts does deserve to be reemphasized; (3) an initiative of creating international data bank of herbs and traditional medicines toxicity shall be discussed and moved forward.

TRADITIONAL MEDICINE RELATED POISONING

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Traditional medicine (TM) included herbal medicine is returning to be a new therapeutic choice of treatment in all over the world as well as Thailand. It is also a venue to be self-dependent in health care. There are now numerous registered TM unregistered products in the market. TMs generally are classified as low toxicity substances, however, poisoning related to them are subsequently recognized and reported. The causes of TM related poisonings include the products, practitioners and patients. Problems related to products' quality include contamination, wrong ingredient and adulteration. Some drugs such as corticosteroids and non-steroidal antiinflammatory are intentional added into some fake recipes. Educational system for TTM practitioners is established. Licensing is also applied. However, some folks who are not in the system are still practicing. By inappropriate dispensing, their recipes would cause toxicity. Patients, themselves, are a cause of poisoning. Some Thai patients seek TM treatment while taking the conventional medicines without notification to their physicians. Drug interaction or additive drug effects sometimes occur and cause drug toxicity. Diagnosis TM related poisoning is also difficult. Inadequate labeling and information regarding to the ingredients are the obstacles for this matter. Patients sometimes are not aware about their toxicity and do not report to their physicians. Awareness is the key to recognize the poisoning from these products. Authorized agency should have proper process for registration of these products. Quality control of the manufacturing should be also strengthened. These measurements would prevent or reduce risks of poisoning by these products. Lastly, public should be educated to be aware of the chances of toxicity from these products.Conclusion: TM has both good and bad sides. Poisoning related to them may be caused by the products, practitioners and patients. Awareness of its toxicity and quality control are the keys for preventing poisoning.

GENE TIMES ENVIRONMENT: CONSIDERATIONS IN IDENTIFYING AT RISK FAMILIES IN AGRICULTURAL COMMUNITIES

Dr. Elaine M. Faustman

Understanding gene-environment interactions, which define susceptibility to chemicals, requires characterization of both exposures to such chemicals and the genetics that determine responses to them. To better elucidate these interactions related to children's health, we can build upon the decade-long longitudinal cohort studies from the NIEHS and EPA supported Children's Health Centers (CHC) and we can translate these lessons learned to inform the National Children's Study design and implementation. We need to use a life-stage specific framework to look at these differences and their relevance to identify neurodevelopmental impacts. Organophosphate pesticides (OPs) can pose occupational health hazards for farmworkers. Variation in the genes responsible for activating OPs to the active oxon form can impact formation of the oxon intermediate and thereby potentially affect cholinesterase inhibition. Our study involves farmworkers and non-farmworkers in the Yakima Valley, Washington State. Biological samples were collected during times of pesticide application and non-spray periods. Urine was analyzed for the non-specific OP metabolites and field test kits were used to measure red blood cell acetylcholinesterase (AChE) activity. DNA was isolated from buffy coat preparations and used for genotyping. We previously established a positive linear relationship between the OP azinphos methyl in blood or urinary dimethylthiophosphate levels and AChE inhibition. We investigated if genotypes for key CYP450 genes might modify this relationship. Our analysis identified variation in the CYP3A5 gene (6986A>G, rs776746). PBPK modeling will determine if genotypic variation at this locus could be used to identify individuals who may be more sensitive to OP-associated effects. A seminal lesson learned from the gene-environmental studies from CHC has been the need to consider the dynamics of the gene-environment interaction. Although frequently we consider genetics to be "stable", recent findings illustrate the importance of considering temporal onset of genetic differences for translating information for the identification of populations at risk.

DRUG/XENOBIOTIC METABOLIZING ENZYME POLYMORPHISMS IN THE CLINICAL OUTCOME IN LUNG CANCER

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Lung cancer is an increasing worldwide public health problem particularly in men. The response to chemotherapy particularly for patients with non small cell lung carcinoma (NSCLC) has been reported to be rather poor. Thus, the investigation of the reasons behind this failure of chemotherapy and thus poorer survival in these patients is very important. It is well known that majority of lung cancer patients are cigarette smokers. Cigarette smoke has been reported to cause elevated levels of carcinogen DNA-adducts which in turn form aggressive tumors by mutating and thus inactivating tumor suppressor genes and thereby increase cancer risk and decrease the survival rates of patients with NSCLC. Carcinogens in cigarette smoke are activated by cytochrome P450s (CYP)s and inactivated by Glutathione S-transferases (GST)s. These enzymes also play an important role in the metabolism of a number of chemotherapeutic agents and thus involve in the drug efficacy, toxicity and resistance in cancer chemotherapy. CYP and GST genes are also found to be polymorphic and their polymorphisms are associated with changes or loss in enzyme activity. In addition, CYP and GST genotypes can be associated with higher mutation frequencies of p53 and K-ras genes which may lead to more aggressive tumour phenotypes**.** Hence, CYP and GST genotypes may have influence on the response to chemotherapeutic drugs and survival of the lung cancer patients. Herein, recent findings with respect to the role of these gene polymorphisms in response to chemotherapy and survival in lung cancer patients will be evaluated.

ROLE OF DRUG-METABOLIZING ENZYMES IN DRUG-INDUCED TOXICITY: INFLUENCE OF MICRORNA AND IMMUE-RELATED FACTORS

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MicroRNAs (miRNAs) are a large family of non-coding RNAs that are evolutionarily conserved, endogenous, and 21-23 nucleotides in length. MiRNAs regulate gene expression by targeting messenger RNAs (mRNAs) by binding to complementary regions of transcripts to repress their translation or mRNA degradation. More than 1,000 human miRNAs have been identified so far. MiRNAs are predicted to target ~60% of human mRNAs and are expressed in all animal cells and have fundamental roles in cellular responses to xenobiotic stresses, which affect a large range of physiological processes such as development, immune responses, metabolism, tumor formation as well as toxicological outcomes. Recently, many reports concerning miRNAs related to cancer have been published, however, the miRNA research in the metabolism of xenobiotics and endobiotics and in toxicology has only recently been established. The expression of drug- and xenobiotic-metabolizing enzymes and nuclear receptors and their regulation by miRNA could be important factors for the outcomes of toxicity and clinical adverse drug reactions. Members of the cytochrome P450 (P450, CYP) family are the most important enzymes catalizing the metabolism of xenobiotics including drugs, environmental chemicals and carcinogens. The different profiles of the expression of P450 isoenzymes determine the amount of reactive intermediates formed and the resulting toxic response. P450 has also been implicated in the bioactivation of procarcinogens to their ultimate carcinogens. Recently, some P450s, such as CYP1B1 and CYP2E1, and nuclear receptors, such as AhR, ARNT, FXR, HNF4 α, PPAR α, PXR, and VDR, have been found to be post-transcriptionally regulated by miRNAs. In the presentation, miRNAs with reference to potential modulation of toxicology-related changes of miRNA expression, role of miRNA in immune-mediated drug-induced liver injury, miRNA in plasma as potential toxicological biomarkers and relevance of miRNA-related genetic polymorphisms will be mentioned.

GENETIC POLYMORPHISMS AND BREAST CANCER SUSCEPTIBILITY IN THAI WOMEN

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Breast cancer is the most common cancer among Thai women. There are evidences that the occurrence of breast cancer will continuously increase in Thailand. Although well established risk factors, such as age at first child's birth, nulliparity, and family history of breast cancer, however, the majority of risk factors are still unknown. Inherited differences in the capacity to metabolize environmental carcinogens have recently been suggested to modify individual susceptibility to breast cancer. We conducted a case-control study in Thai women to evaluate the potential modifying role of genetic polymorphisms of selected low penetrance genes that are involved in estrogen synthesis and metabolism (*CYP1A1, CYP1A2, CYP1B1, CYP17, CYP19, CYP2C9, CYP2C19, ESR1, PGR, ERRG, COMT, NOO1, HSD17B1, HSD17B2, AR*), carcinogen metabolism (*GSTM1, GSTP1, CYP2E1,NAT1, NAT2, ADH, ALDH2*), DNA repair (*hOGG1, APE1, XRCC1, XRCC2, XRCC3*). Genetic polymorphism were determined by PCR, PCR-RFLP, light-cycler and 5'-nuclease assay (Taqman). Some SNPs showed significantly different distribution between case and control. For example, we found that *CYP1A2*, ESR*1*, *NAT2* genotypes were associated with an increased risk of breast cancer, while a protective effect of *CYP1A1, ERRG CYP2E1* genotype were found. Variants leading to reduced or increased enzymatic activity as compared to the wild-type. Our results suggest that genetic polymorphisms in folate and alcohol metabolic pathway influence the risk of breast cancer. Increased risk was observed for homozygotes at the *MTR*. Furthermore, SNPs of the genes that contribute to alcohol behavior, *DRD3*, *DRD2* and *SLC6A4* were also associated with an increased risk of breast cancer. A stratified analysis suggested that the association between these SNPs and breast cancer is different by menopausal status. These results suggest that genetic polymorphisms of some selected penetrance genes in individual susceptibility to breast cancer development in Thai Women and could provide targets for future development of therapeutics.

THE ROLE OF CLINICAL TOXICOLOGIST IN THE MANAGEMENT OF ACUTE AND/OR CHRONIC POISONING OUTBREAKS: EXPERIENCE OF THE TAIWAN NATIONAL POISON CONTROL CENTER

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Background: Clinical (medical) toxicology is a unique and complex specialty focusing on the diagnosis, management and prevention of various poisonings. Clinical toxicologists have the expertise to identify and treat conditions such as acute and chronic effects of different toxic exposures, adverse drug reactions, envenomations, workplace or environmental chemical exposures, criminal poisonings, and other toxicologic problems. Moreover, clinical toxicologists play an important role in the early detection and prevention of poisoning outbreaks. Taiwan National Poison Control Center (PCC-Taiwan) was founded in 1985 and has been involved in the management of numerous poisoning outbreaks in Taiwan. The practical experience of PCC-Taiwan accrued over the last two decades is thus extremely valuable for all healthcare professionals who may encounter poisoning outbreaks in their clinical practice.

Case presentations: Case studies that will be presented in this lecture include outbreaks of both acute and chronic poisoning exposures in Taiwan. Acute poisoning outbreaks that will be presented comprise mollusk related tetrodotoxin poisoning, *Sauropus androgynus* (weight-losing vegetable) poisoning, homicidal carbamate insecticide (methomyl) poisoning, paramethoxy-methamphetamine poisoning, lean meat powder (clenbuterol plus salbutamol) poisoning, and homicidal ethylene chlorohydrin poisoning; whereas chronic poisoning outbreaks consist of aristolochic acid containing Chinese herbs related nephropathy, melamine-contaminated food exposure, and plasticizer (i.e. phthalates)-contaminated food exposure. The role of PCC-Taiwan in the management of the aforementioned poisoning outbreaks will then be discussed.

Conclusions: Clinical toxicologists can play important roles in the management and prevention of various poisoning outbreaks. Nevertheless, clinical toxicologists need to be well-trained, experienced and knowledgeable so that they can achieve excellent professional performance.

CLINICAL TOXICOLOGY IN SRI LANKA: TRANSLATING RESEARCH INTO PRACTICE

Andrew Dawson

The potential targets for implementing require different levels of communication. The most effective target is regulatory change such as pesticide restriction. This requires clear communication between all the partners in the process. At a clinical level evidence based guidelines are frequently produced, the challenge is for them to be utlised especially in remote hospitals.Within Sri Lanka interventions have occurred at a number of levels including undergraduate curriculum review, development and delivery of distance postgraduate clinical toxicology courses. The greatest challenge is influencing existing practice. Variables that influence evidence translation into practice include; the patient and communities prior beliefs about treatment, within the hospital it includes doctors, nurses and other staff. Moving these guidelines into practice is one of the areas of our current research. Issues identified in rural hospitals include; professional isolation, poor access to knowledge on best practice, lack of support to adopt best practice, inadequate stocking of antidotes, inappropriate inter-hospital transfer and competing beliefs of other staff (nursing and attendants) and the community as to what constituted appropriate treatment. We completed a randomized control trial in 44 primary rural hospitals that focused upon initial care in particular decontamination and currently have a further trial in 104 hospitals looking at treatment practices.In our original trial we delivered a short interactive teaching session using a lecture/workshop format in the primary hospital to medical and other hospital staff. This was combined with the distribution of posters describing treatment algorithms derived from National Poison Treatment Guidelines Book plus distribution of promotional items with reminder messages. The results of this study showed that such an intervention could significantly alter some treatment behavior such as increased utilisation of activated charcoal but not alter other behaviors (such as induced emesis). Sociological analysis showed that different staff were responsible for different aspects of treatment, specifically attendant medical staff would induce emesis as they believed that the local community expect this treatment.Ultimately moving research into practice requires a system of evidence communication that clinicians trust.

POISONING ISSUES IN VIETNAM

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Introduction: Poison control is an important part in the health care system. In developing countries, the morbidity and mortality of poisoning are higher than those in developed countries even though these are generally preventable and treated with good outcomes. The poisonings in this area are also more complicated and diverse which are challenges and opportunities to poison control centers. This presentation is to introduce the features of poisoning in the North of Vietnam and experiences of the poison control center in this region.**Content:** Population of Vietnam is 86,927,700 in 2010 with the GDP per capita of 1080 USD approximately. The country has 63 provinces located in 3 regions (North, Centre and South). There are geographical, climate and economical differences among regions. Budget for the healthcare and especially for poison control is very low.Leading causes of poisoning and death rate: agricultural chemical poisoning 22.6% (death 4.92%), venomous animals 15.57% (death 0.93%), drug of abuse 12.84% (death 6.67%), sedative-hypnotics 12.81% (1.51%) and other agents (survey in 44 provinces). The special issues include pesticide poisonings, snakebite, hymenoptera evenomation. The poisoning profile has been changing according to the economical development and social changes. Both classical and newly emerging poisonings can be seen. Classical poisonings e.g. organophosphate poisoning, seizure inducing rodenticide poisoning used to be prominent but now become less frequent. New and emerging poisonings e.g. drug overdoses, drugs of abuse, new pesticide poisonings are more common. At the poison control center, the leading poisoning in 2011 is drug overdose (25.09%), followed by drug of abuse (18.12%), animals (17.68%), pesticides (10.32%), household chemicals (4.67%) and others.The poison control system consists of the 2 Poison control centers, 1 department of clinical toxicology and units of clinical toxicology at departments of emergency or departments of intensive care at all provincial hospitals. Poison control center of Bach Mai hospital: The first department of clinical toxicology was established in 1998 which is separated from the department of emergency and intensive care medicine of Bach Mai hospital. The department became the poison control center of Bach Mai hospital in 2003. The center works as a national poison control center but with very low budget from the government. The human and facility resources are mainly for the clinical toxicology unit which is the most developed unit of the center. The death rate of poisonings at Bach Mai hospital from 8.5% (before the establishment of poison control center) to less than 1% (after the establishment of the center). Many classical poisonings was already resolved in term of the good outcomes, especially organophosphate and nereistoxin poisoning, severe hymenoptera evenomation and snakebite. Researches have been carried out which contributed to the improvement in the care of poisoned patients, e.g. development of gastric lavage set and liquid charcoal preparation, in organophosphate poisoning (death rate currently 1.84% at the center and 3-5% at provincial hospitals), antivenoms and hypertonic sodium chloride in snakebite, continuous venous-venous hemofiltration/hemodialysis and plasma exchange for removal of poisons and supportive care of critically poisoned patients. Toxicology tests including both screening and conformation tests are done at the laboratory of the center. Regarding training and education, clinical toxicology is one important part of emergency and critical care medicine. Learners are trained at the center on the daily basis at the center including medical students, nursing students, emergency and critical care doctors (at all levels). Thousands of learners were trained so far at and outside the center. The center is author and co-author of 23 books in clinical toxicology, emergency and critical care medicine. The center has been being actively working in spite of the current financial crisis because it bases on what it has in hand.The telephone consultation has not been deployed successfully and is now the major limitation in the poison control of Vietnam. **Conclusion:** Poisoning in Vietnam has special features. It creates both difficulties and opportunities for the poison control career. The poison control center has tried it's best to keep the work going only with available resources and brought about certain significant achievements.

FRONTIER OF 3D-CULTURE TECHNOLOGIES FOR EXTRAPOLATING TOXICITY *IN VIVO*

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To create new *in vitro* culture models for extrapolating the cell response *in vivo*, Takezawa *et al.* have attempted to devise culture substrata of anchorage-dependent cells and established five novel 3d-culture technologies.[1-5] Here, I introduce two approaches *in vitro* to predict ADMET (absorption, distribution, metabolism, excretion and toxicity) *in vivo* by applying a collagen vitrigel membrane and TOSHI (tissue/organ sections for histopathology) to the scaffold in 3d-culture.The preparation method of a collagen vitrigel membrane was developed by three processes; the gelation process in which a type-I collagen sol forms an opaque and soft gel, the vitrification process in which the gel becomes a rigid material by the sufficient drying-out, and the rehydration process in which the vitrified material converts into a thin and transparent gel membrane with enhanced gel strength by the moisture supplement. Such a collagen vitrigel membrane is composed of high density collagen fibrils equivalent to connective tissues *in vivo* and is easily handled with tweezers. Also, it possesses excellent transparency and permeability of protein with high molecular weight and consequently the various researches utilizing it as a 3d-culture scaffold advances so well.[6] Especially, the collagen vitrigel membrane chamber is useful for reconstructing culture models such as "tissue sheet" composed of epithelial cells alone or endothelial cells alone and "organoid plate" composed of epithelial cells, mesenchymal cells and endothelial cells.[7] So, I briefly intend to present novel culture models promoted in my laboratory aiming for an eye irritancy test[8] and estimation systems of vascular permeability and hepatic metabolism.On the other hand, TOSHI retains the original microarchitecture and composition of tissues/organs *in vivo*. So, TOSHI can provide signaling cues for inducing cell behavior and phenotypes towards the cells in culture using it as a scaffold. Meanwhile, it is reported that mouse embryonic stem (ES) cells injected into the tail veins of carbon tetrachloride (CCl_4) liver-injured mouse were differentiated into hepatocyte-like cells in the host liver. Therefore, we investigated whether the ES cells could also be differentiated into hepatocyte-like cells when they were cultured on the TOSHI-substrata prepared from livers in various stages after CCl_4 administration into mice. Consequently, it was found that the substrata derived from regenerating livers enhanced cell attachment, supported growth as clusters, and induced differentiation into cells expressing albumin, although the substrata from injured livers did not. In particular, the cells cultured on the most proliferative regenerating liver-derived substratum reconstructed the hepatic cord-like structures with bile canaliculus-like aspects in which some binucleated cells were involved, secreted albumin, and expressed cytochrome P450IA1 activity within a few days.[9] The above data suggest two possibilities for applying the culture system utilizing TOSHI-substrata to the research in toxicology *in vitro*. It would be available as an efficient preparation of mature hepatocytes from stem cells. Also, cell behavior cultured on the TOSHI–substrata derived from animals after drug administration seems to reflect the drug toxicity *in vivo*, and consequently it would provide a novel strategy to extrapolate toxicity *in vivo*.[10]

RISK21

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RISK21 is a program organized by the ILSI Health and Environmental Sciences Institute (HESI) with involvement of scientists from academia, government, and industry from North America and Europe. Its goal is to develop a scientifically based process that has the potential of incorporating the newer advances in computational and molecular toxicology and including appropriate considerations of use and exposure to form the basis of an integrated evaluation strategy to assess risk. It is a fundamental change in philosophy from performing a standard battery of toxicological testing and then thinking about risk assessment to first formulating the problem that needs to be addressed, then selecting sources of information which will have the most value in addressing the problem. Starting with problem formulation, exposure estimates are made rather than relying first on toxicity hazard data. Extensive use of prior knowledge and predictive network analysis is used to identify gaps in information, how best to provide information to fill those gaps and to use probability distributions to characterize human safety. RISK21 principles have been designed to bring applicable, accurate, and resource appropriate approaches to the evolving world of human health risk assessment. RISK21's problem formulation-driven, iterative process identifies data gaps and refines knowledge to the necessary level of certainty to make relevant human health safety decisions.

A NOVEL BIOMARKER TO DETECT ENDOCRINE DISRUPTING CHEMICALS FOR RISK ASSESSMENT: DO THEY HAVE AN ESTROGENICITY OR TUMORIGENICITY IN CELLULAR MODELS?

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The class of endocrine disrupting chemicals (EDCs) indicates both naturally occurring and man-made compounds, which can interfere with the actions of an endogenous steroid. One of estrogens in the body, 17beta-estradiol (E2), is a pleiotropic hormone which regulates the growth and differentiation of many tissues and also acts as a mitogen that promotes the development and proliferation of hormone-responsive cancers. In this study, we examined the alterations of cell cycle-related genes following treatments with diverse EDCs, i.e., bisphenol A (BPA), methoxychlor (MXC), phthalate, benzophenone-1 (BP-1), in estrogen receptor (ER) expressing cells, i.e., breast (MCF-7), prostate (LNCaP) and ovarian (BG-1) cancer cells. These EDCs induced up-regulation of cyclin D1 and down-regulation of p21 in these cells. To determine whether EDCs increase the growth of ER-expressing cells through an ER signaling pathway, we co-treated these cells with agonists of ER signaling pathways (propyl pyrazoletriol, PPT, and diarylpropionitrile, DPN) or an antagonist of ER (ICI 182,780) in the presence of these EDCs. The effect of BPA on the breast cancer cell growth was enhanced in the presence of PPT in MCF-7 cells. The expressions of *cyclin D1* and *p21* were altered by various EDCs in the presence of PPT, but it was less altered by them in the presence of DPN. We knockdowned ERalpha gene expression via siRNA in MCF-7 cells before EDCs treatment. When the expression level of ERalpha was knockdowned in MCF-7 cells, effects of BPA and MXC were lost on the expression levels of *cyclin D1* and *p21* genes. Taken together, these results indicate that various EDCs altered the gene expressions associated with cell cycle, especially in G1/S transition, and resulted in the stimulation of ER expressing cancer cell growth via an ERalpha signaling pathway**.** These collective results confirm that these EDCs may have the carcinogenicity via an ER-mediated signaling pathway *in vitro*. A further *in vivo* study is under investigation to determine whether diverse EDCs may have a potency to be carcinogenic in various human cancer cells. [This work was supported by a National Research Foundation of Korea (NRF) grant funded by the Ministry of Education, Science and Technology (MEST) of Korea government (no. 2011-0015385)]

Keywords: Endocrine disrupting chemicals, estrogen, estrogen receptor, cell cycle, cell cycle related genes, tumor progression

NEW APPROACHES IN IMMUNOTOXICOLOGY

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The realization, that the immune system can be the target of many chemicals including environmental contaminants and drugs with potentially adverse effects on the host's health, has raised serious concerns within the public and the regulatory agencies.The assessment of immunotoxic effects relies on different animal models and several assays have been proposed to characterize immunosuppression and sensitization. The use of whole animals, however, presents many secondary issues, such as expense, ethical concerns, political and practical resistance and eventual relevance to risk assessment for humans. Although formally validated alternative in vitro tests to assess immunotoxicity do not exist, significant progress has been made toward in vitro assays in the last decades. Alternative in vitro assays to detect immunosuppression and allergic hypersensitivity have the potential to reduce animal use, testing cost, and to increase throughput of immunotoxicity screening and prioritization efforts. Therefore, such models can be used for the pre-screening and hazard identification of unintended immunosuppression and contact hypersensitivity of direct immunotoxicants. This presentation intends to review the state-of-the-art in the field of in vitro immunotoxicity.

KEFIRAN REDUCES ATHEROSCLEROSIS IN RABBITS FED A HIGH CHOLESTEROL DIET

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Aim: Kefiran is an exopolysaccharide produced by Lactobacillus kefiranofaciens, and has been pro- posed to have many health-promoting properties. We investigated the antiatherogenic effect of kefi- ran on rabbits fed a high-cholesterol diet. **Methods**: Male New Zealand White rabbits were fed a 0.5% cholesterol diet without (control group, n=7) or with kefiran (kefiran group, n=8) for eight weeks. The aorta was analyzed by histochemis- try and atherosclerotic lesions were quantified. Lipids and sugars in serum were measured. Foam cell formation of RAW264.7 by βVLDL derived from both groups of rabbits was also investigated. **Results**: Cholesterol, triglyceride and phospholipids levels of serum and lipoprotein fractions were not significantly different between these groups. Atherosclerotic lesions of the aorta in the kefiran group were statistically lower than those of the control group, with marked differences in the abdominal aorta. T-lymphocytes were not detectable in the aorta of the kefiran group. Cholesterol contents in stools were almost identical in both groups. Cholesterol content in the liver of the kefiran group was statistically lower than in the control group. Galactose content of βVLDL derived from the kefiran group was higher, and the lipid peroxidation level was much lower than in the control group. RAW264.7 macro- phages treated with βVLDL from the kefiran group showed a more spherical shape and accumulated statistically lower cholesterol than macrophages treated with βVLDL from the control group. **Conclusion**: Orally derived kefiran is absorbed in the blood. Kefiran prevents the onset and develop- ment of atherosclerosis in hypercholesterolemic rabbits by anti-inflammatory and anti-oxidant actions.

EFFECTS OF LIGNANS FROM SESAME SEED ON GENOMIC AND NON-GENOMIC ESTROGEN SIGNALING

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Currently, uses of phytoestrogen-rich natural products as hormone supplement and chemopreventive agents are increasing. In postmenopausal women, production of estrogen from ovary ceases leading to several health impacts such as hot flashes, osteoporosis, fatigue, and impaired cognition. A number of medicinal plants and several foods such as soy products, flax seed, and dried legume are considered to be nutraceutical chemopreventive agents as they contain phytoestrogens. Sesamin and sesamolin are oil soluble lignans from sesame seeds while sesamol is a phenolic compound derived from conversion of sesamolin during the roasting of sesame seed and processing sesame oil. The three compounds from sesame seeds stimulate genomic estrogen signaling as evidenced by induction of estrogen responsive element (ERE)-dependent transcriptional activation of luciferase reporter and induction of estrogen-regulated progesterone receptor (*PGR*) and *pS2* genes. The activation of genomic estrogen signaling by these compounds is mediated via estrogen receptor (ER) as the antiestrogen ICI 182 780 can abolish these effects. Estrogen and its signaling are known to play a crucial role in development and progression of hormone-dependent breast cancer which the cancer cells express ER subtype alpha (ERα +ve) but not hormone-independent breast cancer (ERα -ve). Use of phytoestrogen as chemopreventive agent in hormone-dependent breast cancer is of concern as it may stimulate tumor growth. We demonstrated that sesamin, sesamolin, and sesamol partially inhibited *in vitro* growth of hormone-dependent T47D human breast cancer cells while sesamol dose-dependently inhibited growth of hormone-independent MDA-MB231 human breast cancer cells. Interestingly, the three sesame seed compounds induced growth of T47D cells when cultured in estrogen-withdrawal (E_2W) medium which mimic post-menopausal condition. Additional to the classical genomic ER signaling, the rapid non-genomic membrane ER (mER) and GPR30/EGFR/MAPK signalings are recently found to be involved in regulation of growth and stress response of cells. Sesamol rapidly induced phosphorylation of EGFR and MAPK in E_2W condition suggested that in addition to genomic ER it may also activate the rapid non-genomic ER signaling.Taken together, our studies demonstrate that sesamin, sesamolin, and sesamol from sesame seed are phytoestrogens. Their weak estrogenic properties should be of benefit as a hormonal supplement in postmenopausal women. However, precautions should be taken in postmenopausal women with hormone-dependent breast cancer.

HEALTH BENEFICIAL EFFECTS OF PHYTOPHARMACEUTICALS AND PHYTONUTRIENTS: XENOHORMETIC PERSPECTIVES

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A wide variety of phytochemicals present in our diet, including fruits, vegetables, and spices, have been shown to possess a broad range of health-beneficial properties. The cytoprotective and restorative effects of dietary phytochemicals are likely to result from the modulation of several distinct cellular signal transduction pathways. Phytopharmaceuticals/ phytonutrients, either alone or in combination, maintain a precise control over cellular redox status by suppressing activation of various upstream kinases, their downstream transcription factors and their regulators. From an evolutionary perspectives, many dietary phytochemicals that are synthesized as secondary metabolites function as toxins, that is, "phytoalexins," and hence protect the plants against insects and other damaging organisms, microbial infection and stresses. However, at the relatively low doses consumed by humans and other mammals these same “toxic” plant-derived chemicals activate adaptive cellular stress response signal transduction pathways, conferring stress resistance and other health benefits. This phenomenon has been referred to as xenohormesis. One of the key players responsible for the the xenohormesis mechanisms underlying cytoprotective effects of some dietary phytochemicals is the nuclear transcription factor erythroid 2p45 (NF-E2)-related factor 2 (Nrf2) that has been evolutionarily conserved in diverse species

SAFETY RE-EVALUATION OF CHINESE TRADITIONAL MEDICINE IN INJECTION FORMULATION

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Lianbizhi Injection was developed as a single component of Traditional Chinese Medicine(TCM)injections in 1970s. With the increased clinical applications, the relevant reports of adverse reactions were increased. Therefore, we carried out safety re-evaluation of the Lianbizhi Injection in this program, based on the guiding spirit of the SFDA note for the industry, and on the adverse reactions of Lianbizhi Injections reported in clinicals. Firstly, the general toxicity tests such as acute toxicity test, single dose kidney toxicity test, 30-day repeated dose toxicity test and allergy test had been conducted to explore the toxicity of two kinds of formulations of Lianbizhi Injections (the concentrations of Andrographolidi Natrii Bisulfis for Lianbizhi Injections A and B were 235.5 and 117.4mg/ml, respectively, with relevant impurities contents being 1.3% and 50.9%, respectively). Secondly, the nephrotoxicity mechanisms are explored, by using modern metabonomic technologies, *in vitro* cytotoxicity test and the combination effect study with of kanamycin. The results of studies *in vivo* and *in vitro* confirmed that Lianbizhi Injections had potential nephrotoxicity in high concentration. The mechanism of renal injury induced by Lianbizhi Injections may be related to affecting the osmolality in kidney medulla and interference the activity of related mitochondria enzymes in renal cell, involving in affecting mitochondria energy metabolism, changing the redox state, resulting in excessive ROS, destructing the cell membrane permeability, declining MMP, mitochondrial dysfunction, leading to CytC releasing from mitochondria to trigger apoptosis or necrosis. The toxic effects will occur when Lianbizhi Injections was used in large dose and unreasonable combination therapy. The related substances in the preparation may have great influence on the safety of Lianbizhi Injections.

Keywords: Lianbizhi Injection, Andrographolidi natrii bisulfis, Safety re-evaluation, toxicity, metabonomics

BURDEN OF DISEASE ATTRIBUTABLE TO CHEMICALS AND AIR POLLUTION: OVERVIEW OF THE EVIDENCE

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Chemicals, whether of natural origin or produced by anthropogenic activities, form part of our environment. Exposure to chemicals, including through air, water, food, soil and products have the potential to impact on health world-wide. The health impact associated with chemicals was estimated by WHO using standard Global Burden of Disease (BOD) methodology in which the disease burden is expressed in number of deaths as well as in Disability-Adjusted Life Years (DALYs), the number of years lost due to ill-health, disability or early death. BOD estimates were developed for a number of chemicals and chemical groups, including (a) chemicals involved in unintentional acute poisonings, (b) chemicals involved in unintentional occupational poisonings, (c) pesticides involved in self-inflicted injuries, (d) asbestos, (e) occupational lung carcinogens, (f) occupational leukaemogens, (g) occupational particulates, (h) outdoor air pollutants, (i) indoor air pollutants from solid fuel combustion, (j) second-hand smoke, (k) lead, and (l) arsenic in drinking water. The study showed that in 2004, 4.9 million deaths (8.3% of total) and 86 million DALYs (5.7% of total) were attributable to exposure to chemicals and chemical groups involved in the WHO study. The largest contributors to the global BOD include indoor smoke from solid fuel use, outdoor air pollution and second-hand smoke, with 2.0, 1.2 and 0.6 million deaths annually. These are followed by occupational particulates, chemicals involved in acute poisonings, and pesticides involved in self-poisonings, with 375,000, 240,000 and 186,000 annual deaths, respectively. Although the known burden due to chemicals is considerable, the WHO study covers only chemicals for which data were available; therefore, the results were more likely an underestimate of the actual burden. Chemicals with known health effects, such as dioxins, cadmium, mercury or chronic exposure to pesticides were not included due to incomplete data and information.

USING EVIDENCE TO PREVENT DEATHS FROM PESTICIDE POISONING IN RURAL ASIA

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Self-poisoning with pesticides is a major clinical problem in rural Asia, killing several hundred thousand people every year. The most important pesticides are organophosphorus insecticides, which are widely used in agriculture and responsible for about 2/3 of deaths. Inhibition of acetylcholinesterase in poisoned humans results in overstimulation of cholinergic synapses in the central nervous system, autonomic nervous system and neuromuscular junction. Deaths occur due to acute respiratory failure, usually before hospital admission, or due to complications of reduced consciousness and respiratory arrest - aspiration pneumonitis and cerebral hypoxia. Over the last 10 years, our clinical toxicology research group has carried out clinical studies in the North Central Province of Sri Lanka to improve treatment and reduce deaths from pesticide poisoning. We have tried to standardise therapy, using the evidence base, and performed randomised controlled trials to test the effectiveness of activated charcoal and pralidoxime. At the same time, using a complementary public health approach and a Haddon matrix as a guide, we have moved out of the hospital and looked for solutions in the community. We have studied the effect of pesticide bans on both human health and agricultural output and are currently assessing the effectiveness of self-storage containers to reduce pesticide poisoning. Our group attempts to develop evidence for possible interventions at multiple levels, including the patient, the community, and government legislation, to ultimately reduce deaths from pesticide poisoning in rural Asia.

REDUCING THE BURDEN OF DISEASE FROM METALS THROUGH REGULATION AND POLICY MEASURES: SUCCESSES AND POSSIBILITIES

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Of the ten chemicals of major public health concern identified by WHO, three are metals: cadmium, lead and mercury. These cause a significant burden of disease through both acute and long-term health impacts. They largely enter the environment and, therefore, human exposure pathways, through anthropogenic activities, particularly mining, smelting, manufacturing, and waste dumping and processing. Measures to protect human health by controlling exposures started in 1921 with the ILO White Lead (Painting) Convention. The growing understanding of the harmful effects of chemicals on health and environment over the last decades has led to the development of a plethora of international conventions and partnerships that provide mechanisms for controlling the use and release of selected chemicals. Examples are the Rotterdam Convention on Prior Informed Consent, the Basel Convention on the Control of Transboundary Movements of Hazardous Waste, the Partnership for Clean Fuels and Vehicles, and the Global Alliance for the Elimination of Lead in Paint. There are, in fact, 7 international conventions that include provisions for lead, 6 for mercury and 5 for cadmium. At national level, regulatory measures such as standards for industrial emissions, occupational safety, and food and drinking water all contribute to reducing exposure to these metals. Banning the use of leaded gasoline has had a dramatic impact on lead exposure; in countries that have taken this action mean blood lead concentrations have shown a decline ranging from 30-48%. Negotiations are currently underway to develop a global, legally binding instrument on mercury that will control the use and release of that metal. While regulation and policy measures have been successful in reducing exposures to toxic metals in many countries, problems still remain. The informal sector remains poorly controlled and, in some countries, is responsible for significant environmental contamination with mercury (artisanal gold mining) and lead (lead battery recycling).

HEALTH IMPACT FROM HAZARDOUS WASTE SITES IN INDIA, INDONESIA, AND THE PHILIPPINES

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Background: Prior calculations of the burden of disease from toxic exposures have not included estimates from hazardous waste sites due to the absence of data on exposures and health effects.**Objective:** To develop a disability adjusted life year (DALY)-based estimate of the morbidity and mortality attributable to hazardous waste sites in India, Indonesia, and the Philippines. **Methods:** This paper utilizes data collected in Blacksmith Institute's Toxic Sites Identification Program (TSIP), an effort to identify contaminated sites in low and middle income countries. Investigators sampled chemical hazards in various environmental media and estimated the human population at risk of exposure at each site. Utilizing published dose-response relationships, we estimated the incidence of disease resulting from these chemical exposures. By combining these incidence estimates with the population at risk, we calculated the DALYs attributable to hazardous waste sites in these countries. **Results:** 9.8 million individuals are at risk of exposure to one of eight industrial pollutants at 435 hazardous waste sites in three countries. These exposures result in approximately 1.67 million DALYs over the course of the exposed individuals' lifetimes, accounting for approximately 0.44% of the total DALYs in these countries. The burden of disease from these hazardous waste sites ranks above the burden from malaria and below the burden from HIV/AIDS.**Conclusions:** Hazardous waste sites in these three countries present a significant burden of disease, highlighting the need for these sites to be remediated and the health of the affected human populations to be monitored.

OCCUPATIONAL EXPOSURE TO MERCURY IN AN OIL AND GAS INDUSTRY: CONFOUNDING EFFECTS OF SEAFOOD INTAKE IN BIOMONITORING OF BLOOD

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Organomercurials are generally found in fish and shellfish whereas elemental mercury if present in crude oil and natural gas at some hotspot areas. In this study, the toxicological health risk assessment of mercury (Hg) was conducted on oil and gas personnel and contractors who were involved in 13 major and minor shutdowns. All databases related to Hg activities between 2006-2010 were utilized for analysis of potential sources of exposure through air, water and food. Furthermore, the levels of Hg in condensate, crude oil, food from the galley and in personnel's blood and urine were investigated. The species of Hg present in the condensate and crude oil was primarily elemental but organic Hg was predominantly found to be present in fish at the galley. Hg was not detected in the water and this route of exposure is not significant for the purpose of risk assessment. Preshift and postshift blood data from 2671 and 2502 personnel respectively were analyzed for Hg potential exposure. From the preshift total blood Hg, only 78 (2.9%) personnel had Hg level exceeding the Biological Exposure Index (BEI) confirming that the high exposure was unrelated to the workplace but potentially due to dietary intake especially seafood or other sources. In the postshift blood Hg analysis, 94 (3.8%) personnel showed blood Hg above BEI. Following a review of the investigation report, ambient and personnel air monitoring data and blood speciation results, only 13 (0.52%) personnel were confirmed to experience occupational exposure to Hg during the shutdown activities. In contrast, the remaining 81 (3.2%) personnel had Hg exceeding BEI most probably due to ingestion of Hg from the seafood in the galley. Health risk assessment calculation indicates that those personnel categorized as high fish intake group are at risk of exceeding the acceptable daily intake of Hg. Taken together, the results of this risk assessment and the nature of exposure to elemental and inorganic mercury in crude oil and condensate, urine Hg corrected to creatinine level is a better occupational exposure biomarker for the company's personnel since total blood Hg may also reflect exposure from dietary intake.

OCCUPATIONAL AND ENVIRONMENTAL BLADDER CANCER RISK AND GENETIC SUSCEPTIBILITY

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Bladder cancer is a model tumor of chemical carcinogenesis which is, in general, more frequently observed in industrial and urban areas than in rural areas. It can be caused by different risk factors, including occupational and environmental exposure. The second most relevant cause of bladder cancer is exposure to bladder carcinogenic substances in the workplace, mostly carcinogenic aromatic amines and bioavailable (i.e. water soluble) azo dyes that can be cleaved in the body into the carcinogenic aromatic amine. Production and use of azo dyes have been an important issue in developing countries for decades. However, there is a considerable discrepancy between the number of identified occupational-related bladder cancer cases and the estimated numbers particularly in emerging nations or less developed countries. Bladder cancer risk can be modified by genetic factors. The most important genetic factor regarding aromatic amine-related bladder cancer risk, resulting from occupational exposure or tobacco smoke which also contains carcinogenic aromatic amines, is the polymorphic xenobiotic metabolizing enzyme N-acetyltransferase 2 (NAT2). Interestingly, formerly exposed Caucasians presenting the “slow” NAT2-acetylation status have an increased bladder cancer risk whereas formerly exposed Chinese have not. The impact of the glutathione S-transferase M1 (GSTM1) and other polymorphic xenobiotic metabolizing enzymes regarding occupational and environmental exposure is controversial. Another important environmental bladder cancer risk factor is exposure to arsenic via drinking water or, in some areas, inhalation of combustion products from high arsenic coal. It has been shown in several studies that arsenic carcinogenicity is also modulated by single nucleotide polymorphisms (SNPs). Since 2008, several large genome-wide association studies have been performed in bladder cancer cases. Surprisingly, most of the SNPs identified in these studies do not belong to loci known to be associated with increased bladder cancer risk and do not modulate bladder cancer risk resulting from occupational exposure or smoking habits. To date, all known genetic risk factor can only explain a small part of the bladder cancer risk. Occupational exposure, smoking habits, SNPs as well as bladder cancer incidence may vary considerably between different countries. This is a unique opportunity for toxicologists in developing and developed countries to further elucidate bladder cancer risk via new joint studies in exposed groups in different countries.

PAH AND CRYSTALLINE SILICA DUST EXPOSURE-RELATED HAZARDS IN OCCUPATIONAL ENVIRONMENTS IN TURKEY

Sema BURGAZ

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Exposure to individual and mixtures including polycyclic hydrocarbons (PAH) and crystalline silica occurs worldwide in a large variety of industries and occupations, and have been classified as Group 1 and Group 2A carcinogens by the International Agency for Research on Cancer (IARC). Molecular epidemiological approaches may play a role to identify exposure-related hazards and to evaluate for health risk assessment of carcinogens as well as for governmental regulations. Toxic exposures in the environment including occupational exposures are responsible for a substantial percentage of all cancers. Approximately 2% to 8% of all cancers are thought to be due to occupation. It is known that many cancers caused by occupational exposures can be prevented, however, the aspects of occupational cancers are quite different between high-income and low-and middle–income countries. There have been about 1.320.000 workplaces, nearly all of them comprise small and middle-sized enterprices, and, 10.000.000 compulsory insured workers in our country. Expected number of occupational disease cases is between 44.000 and 118.000 for our country, however ,the number of occupational diseases is only 533 to the Social Security Institute Statistics in 2010, furthermore, there is no precise data on occupational cancer cases attributable to occupational diseases. This speech will focus on molecular epidemiological studies carried out in coke oven workers exposed to PAH and in workers exposed to crystalline silica-containing dust associated with jobs such as grinding, mixing, bagging and sandblasting to address the issues concerning with occupational cancer risk in our country.

RATIONAL USE OF ANIMAL TOXICOLOGY DATA IN SETTING UP OCCUPATIONAL EXPOSURE OF CHEMICALS TO HUMANS

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Chemical companies have long recognized that the development and manufacture of agents can lead to adverse health effects if exposure in the workplace is not adequately controlled. In this talk, the methodology used to predict potential health effects resulting from occupational exposures to chemicals, and approaches to setting health-based occupational exposure limits (OELs) from animal toxicology data will be presented. Toxicological studies are an important part of the pre-clinical safety evaluation of potential of chemicals. This pre-clinical safety evaluation of chemicals is tightly regulated by national agencies such as the US EPA and many data, such as acute and repeat dose toxicity by relevant routes, reproductive toxicity, genotoxicity, carcinogenicity and metabolism, are generated in a variety of animal species. These data, as well as a wealth of human data obtained from accidental occupational exposure are available for the occupational toxicologist to identify potential endpoints of concern as a result of occupational exposure to pharmaceuticals. The development of a toxicological testing programme requires consideration of the potential for occupational exposure, likelihood of causing an adverse effect, availability of compound for testing and probability that the chemical will reach large-scale manufacture. This typically leads to the development of a tiered testing approach that is linked to the development track of the new substance. Data generated from the occupational toxicology test battery are used to establish company OELs or generic exposure control categories defined by hazard categories or bands. The data are also communicated to those potentially handling the chemical, such as company employees, contractors, toll manufacturing partners and distributors, by a variety of hazard communication methods such as safety data sheets, labels and training programmes. The chemical industry has provided leadership in a number of key areas, including: the adoption of tiered approaches to occupational toxicology testing that utilize non-animal predictive methods; the methodology for setting in-house exposure control limits; the design and promotion of the use of performance-based control approaches. Clearly, much of this work has been driven by a real need to control occupational exposures to substances that can have profound adverse health effects in exposed employees.

ROADMAP FOR NANOSAFETY - A MISSION IMPOSSIBLE?

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The use of engineered nanomaterials (ENM) grows rapidly. This has led to a quick increase of human exposure to ENM. However, the amount of knowledge on hazards associated with exposure to ENM, or the levels of exposure to them remain inadequate rendering reliable assessment of their potential health risks a challenge. The uncertainty around the health effects and safety of ENM has become an important bottle-neck preventing investments into innovations that could lead to novel ENM or their applications. At the same time, search for quick and predictive methods for assessing health hazards of ENM has been intensified. This has prompted attempts to assess effects of ENM at molecular and cellular levels by using quick and powerful omics technologies, bioinformatics and systems biology. Furthermore, roadmaps have been developed to guide further research endeavors that would enable solving the challenges associated with ENM safety. The following research priorities for the basis of the developed roadmaps have been identified: 1) to develop means to characterize the bio-identity of various ENM; 2) to identify factors that markedly affect exposure to ENM and affect their transformation during their life-cycle; 3) to identify key-hazard mechanisms leading to ENM toxicity at molecular, cellular, organ and organism levels; and 4) to develop reliable risk prediction tools for ENM. In addition, it will be important to intelligently develop a depository of relevant material characteristics and biological information on ENM to be able to develop quick and affordable means to allow reliable assessment of hazard characteristics of various ENM based on their potential toxicity mechanisms. The goal is to find associations and causalities between various hazards markers of and ENM characteristics. Supported by the European Union 7th Framework Program Grant CP-IP-211464-2 (NANODEVICE Project).

SIZE DEPENDENT TRANSLOCATION PATTERN, CHEMICAL TRANSFORMATION AND TOXICITY OF FERRIC OXIDE NANOPARTICLES IN THE CENTRAL NERVOUS SYSTEM

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The toxic effects or biomedical functions of nanoparticles largely depend on their translocation behaviors. However, because of a large number of variable parameters of nanoparticles, experimental measurements of each type of nanoparticles *in vivo* become a huge work and almost impossible. Thus, establishing theoretical models for rapid analyses will be beneficial to both safety estimation and application of nanotechnology. In the present study, the size-dependent translocation mode and biological fate of intranasally instilled Fe_2O_3 nanoparticles (40 nm and 280 nm) in CNS were investigated. The nanoparticle translocation in different parts of brain at 4h, 12h, 24h, 3d, 7d, and 30d were quantified using ICP-MS technique. A biexponential model (correlation coefficient r=0.98~0.99) was satisfactory to describe the particokinetic translocation behavior of Fe_2O_3 nanoparticles in brain. We found a size-dependent translocation pattern and a size-sensitive time-window (4-72 h) of the nanoparticles in the brain, which are most significant in toxic concerns of nanoparticles in the CNS. The synchrotron-based techniques such as microbeam X ray fluorescence (SR-μXRF) and near-edge X-ray absorption spectroscopy (XANES) were then used to map the spatial microdistribution and to identify the chemical forms of the nanoparticles in the brain, respectively. The SR-μXRF images showed the 40 nm-Fe_2O_3 particles more widely distributed in brain regions than the large Fe_2O_3 particle did. The XANES results indicate that the presence of chemical speciation of the Fe_2O_3 nanoparticle (~15%) and protein-complex like apotransferrin-Fe_2O_3 (~16%) in brain. We further showed that intranasal exposure of 40 nm-Fe_2O_3 particles could lead to pathological alteration in olfactory bulb, hippocampus and striatum, and caused microglial proliferation, activation and recruitment in these areas, especially in olfactory bulb. Last but not the least, all the findings suggest size-sensitive manners of nanoparticles in brain, the smaller one possesses evident detention properties in the CNS vs. the larger one.

TOXICITY TESTING AND INTRACELLULAR UPTAKE OF GOLD NANOPARTICLES

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Noble metal gold nanoparticles scatter and absorb visible light due to their strongly enhanced surface plasmon resonance properties. They are therefore used for optical, photonic as well as biomedical applications. Gold nanoparticles also have fluorescence quenching or enhancement properties of organic dyes due to the resonant energy transfer (RET) between organic dye molecules and gold nanoparticles. When assessing the toxicity of gold nanoparticles, conflicting results are reported as to their toxicity when using conventional toxicity tests which rely on fluorometric, colorimetric, and luminometric changes of their final products. Indeed, in our investigations similar contradictory results were obtained when we implemented toxicity tests including LDH, XTT, and ATP toxicity assays. We used these tests to evaluate the toxicity of naked gold nanoparticles as well as those with different functional groups on bronchial epithelial cell line (BEAS-2B) and human embryonic kidney (HEK293) cells *in vitro*. To overcome the interference in these conventional assay systems by the tested gold nanoparticles, we have used the xCELLigence system, a non- invasive and label free system. The normalised cell index (CI), an indicator of the level of adhesion and therefore viability of the cells, were then determined for BEAS-2B and HEK293 cells following their exposure to different concentrations of citrate capped 14 nm gold nanoparticles as well as to 14 nm gold nanoparticles with monolayer protected clusters (MPCs) with different functional groups (PEG-OH, PEG-COOH, PEG-Biotin, PEG-AZ,). The concentration at which least toxicity was observed was then used to assess cellular internalization using the CytoViva Hyperspectral systems. Using these two technologies, it was possible for us to confirm the non toxicity of 14 nm citrate capped gold nanoparticles in the two cell systems with a difference in that these gold nanoparticles entered the BEAS-2B cells at a much faster rate than into the HEK293 cells. The different gold MPCs on the other hand have shown dose-dependent toxicity as well as different cell uptake rates into these two cell types in culture. It is therefore imperative that when using noble metal nanoparticles, attention should be paid to their interfering properties with the dye material used in these toxicity assay systems.

NATIONAL NANOSAFETY AND ETHICS STRATEGIC PLAN: THAILAND'S PERSPECTIVE

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Since the new dawn of nanotechnology era, Thailand has realized the importance of this emerging technology and has been applying nanotechnology in various industries, i.e. textiles, plastics, coating and paint, and cosmeceutical industries. A number of nano-products, for examples, water repellent clothes, antimicrobial coated materials, nano-encapsulated vitamins and nutrition, nano-emulsion skin care products, are commercially available. Nonetheless, nanosafety guidelines are needed to gain public acceptance and to maintain sustainable development. Thailand has taken a leading role in the nanosafety and risk management of nanotechnology. The National Nanotechnology Center (NANOTEC), National Science and Technology Development Agency (NSTDA) has initiated the "National Nanosafety and Ethics Strategic Plan", to be approved by the cabinet, to provide the strategy and guideline at the national level. This includes nanosafety and ethical issues covering education, regulation, monitoring and management. The scopes of the National Strategic Plan are categorized into three strategies:

1. To establish the knowledge management system to collect, analyze, utilize and distribute the information of nanosafety and ethics of nanoproducts
2. To develop and reinforce measures as well as mechanisms of monitoring and enforcement
3. To promote public engagement

These strategies will support and monitor health and environmental impacts of nanotechnology in Thailand. Importantly, three nanosafety guidelines have been established for the public, for manufacturers, and for researchers. The database of nanotechnology information is also available on website: www.knownano.org. Moreover, a nano-labeling system, called NanoQ, is being established by the Nanotechnology Association of Thailand, in cooperation with NANOTEC, universities, regulators and industries. NanoQ is a certification mark given to approved nano-products containing nanomaterials or nanotechnological processes.

PROGRESS IN THE EUROPEAN CHEMICALS LEGISLATION REACH AND IMPACT ON TOXICOLOGY

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Industrial chemicals with inadequate safety data sets may pose a risk for employees, consumers and the environment. However, industrial chemicals are urgently needed for chemical synthesis or technical purposes. Any risk for human health and environment need to be addressed in advance of use or distribution. The chemical legislation of the EU, *Registration, Evaluation and Authorization of Chemicals* (REACH) forces the responsibility of manufacturers and importers of chemical substances to gather the information needed to define the right circumstances of use and control of chemical substances and products. Producers, importers and down stream users have to compile and communicate standard information for all chemicals produced or imported in quantities beyond 1 t/a. These standard data sets are designed to guarantee adequate handling in the production chain, in transport and use. The rules for handling shall prevent the substances from being released to and distributed within the environment. By December 2010 more than 23000 registrations have been submitted for the first group. They cover safety information for more than 4100 substances.A new independent institution, the European Chemical Agency (ECHA) has been established in order to ensure effective management on community level. The Agency shall undertake all tasks related to the registration, the management of dossiers and the coordination of the evaluation of chemicals. For practical reason such as limitations in time and resources legal decision need to be undertaken before the whole set of data can be gathered together for all chemicals that are produced and are needed for specific purposes. Hazards of chemicals can be assessed within certain limits on the basis of acquired knowledge and may give valuable insights and arguments to decide on the priority whether full risk assessment is needed. The innovations under REACH will introduce some flexibility, such as waiving of standard tests and simplification of administrative procedures, in order to gain resources. REACH focus also on new approaches to adapt standard testing regimes in order to foster a risk oriented approach in required work load, to decrease animal based tests and to strengthen weight of evidence in order to avoid unnecessary “tick box testing “.Emphasis will be laid on assessment of exposure which has strong impact to decide on the need of testing. Computational based modelling, animal free based testing and category approaches are also part of waiving options. Read across is applied as part in risk assessment. The new chemical has to have reasonable structural similarity with chemicals used for comparison in this evaluation. The technical guidelines need to be specified in advance and applied by qualified and experienced persons. Read across has limitation because it cannot generate new insights on complex pathways of toxic action. Furthermore, it is critically dependent upon the enzymic pathways involved, on species-specific differences in enzyme patterns and interaction of chemicals in mixtures. Read across will support to manage the work load in chemicals risk assessment but will generate uncertainties of their own. Therefore these limits of acceptance need to defined before the work is undertaken. Members of the scientific community will have to define their own position as researchers, teachers and experts in order to support the efforts to protect human health and environment. Novel techniques which need further work and may offer new insights are in particular RNA expression profiling, proteome analyses and metabonomics to describe alteration in gene or protein expressions patterns or metabolite concentrations in response to toxic stimuli.

PESTICIDE REGULATIONS

Professor Lewis L Smith

Pesticides are now amongst the most intensely regulated substances in the world. This regulation is based on the efficacy, environmental impact and health effects of the individual pesticide and the intention of this presentation is to concentrate on the evaluation and regulation of health effects. Globally, this regulation has been established on an individual country basis, but in some regions, such as the European Union (EU) a two-tier process involving the approval of individual pesticides by the EU (Annex 1 listed) is followed by regulatory approval of those Annex 1 listed pesticides in individual European countries. Also, within the USA the Federal registration of pesticides by the Environmental Protection Agency (EPA) is subject to modification and approval by individual states.Many nations in the developed and developing world have well established independent, technical review processes for the regulation of pesticides. However, although WHO and FAO provide mechanism for the co-ordination of decisions amongst groups of countries, the development of appropriate skill bases for the sound toxicological and environmental impact of pesticides is seriously lacking in many developing countries. Furthermore, irrespective of the sophistication of the regulatory processes in individual countries or regions of the world, without appropriate policing of regulations, training of applicators and general education of the user population, there is the likelihood of continued misuse or abuse of pesticides.The regulation themselves are usually based on a risk/benefit analysis. However, since the benefit is often enjoyed by those not exposed to pesticides, the emphasis on risk is rightly balanced on protecting those who are most exposed to the potential hazard; this in effect means spray operators and other farm workers. Importantly, relevant measures of exposure to pesticides is an expanding science and involves, not only those with occupational exposure but the general public who consume food products containing pesticide residues. The development of measures to avoid or reduce exposure necessitates a comprehensive understanding of the life cycle of pesticides.The toxicological test for pesticides covers a range of potential toxicities. Although, there is a regulatory framework for these tests, the manufacturer has a duty of care to ensure that appropriate evaluations are carried out that reflect the use, exposure and likely hazards that could arise from an individual pesticide. The major toxicological evaluations are designed to evaluate the effect of pesticides on their acute and chronic toxicity, their mutagenicity and carcinogenicity, and their effect of the reproductive, nervous and the immune systems. These tests are carried out using both in vitro and in vivo techniques but they all require extrapolation to humans. This remains the most critical and often controversial issue in the interpretation of toxicological data. For many toxicologists it is only when mechanism of toxicity of an individual chemical is understood, can a sound and relevant interpretation of toxicological data can be made. In the future the regulation of pesticides is likely to demand a much greater understanding of the relevance of experimental data to the human population.

REGULATORY SCIENCE FOR THE SAFETY EVALUATION IN NON-CLINICAL RESEARCH AND DEVELOPMENT

Ikuo HORII

Pfizer, Drug Safety Research & Development

Regulatory science thought in drug safety evaluation

Generally drug safety evaluation has been carried out from the toxicological aspect by using standardized toxicological studies. However, comprehensive safety evaluation should be widely addressed to diverse disciplinary sciences such as target pharmacology, toxicology, pathology, drug metabolism / pharmacokinetics, and physical chemistry, with taking into consideration of efficacy and side effect.

In order to detect toxic effect, setting out of appropriate toxicological endpoints as a biomarker is the primary trigger for the safety estimation in each corresponding study. Based on the data-endpoints estimation, total scientific evaluation (type of toxicity, mechanism of toxicity, therapeutic index: ratio of efficacy and toxicity, reversibility, human relevance) executes under the discussion about risk assessment / management.

In addition to the approach from traditional diversity science, new science and technology like molecular toxicology have been introduced to have a better understanding for mechanistic interpretation of the toxicity generation.

Regulatory science in drug research & development stage

Concept and content for safety evaluation are different in each stage of drug research & development. Safety evaluation in toxicity studies is mainly focused on IND/NDA for applications and approvals, however recently comprehensive total safety evaluation would be needed to assure the efficacy and safety in human. Instead, particularly in discovery stage of lead-compound optimization and at the timing of the first clinical application of candidate compound, new high level science and technology are introduced for the mechanistic toxicology. Taking all these assessments, we should evaluate the safety with considering its risk assessment / management. Currently following toxicological studies for the safety evaluation in respective stages are classified as follows: (1) screening toxicity test in the early discovery phase, (2) exploratory toxicity study for clinical testing candidate compound selection, (3) toxicity test for the evaluation at the timing of FIH (First-In-Human) and IND (Investigational New Drug), (4) toxicity test for NDA (New Drug Application), (5) post-marketing safety evaluation. Combining these aspects comprehensively, regulatory scientists should be taken into consideration for the final safety evaluation to human application.

Positioning of GLP/Non-GLP and vision of related guideline for safety assessment

In the process of GLP-orientation, there is misleading as if they are in science, despite GLP is in line to assure the quality and integrity of data. Thinking scientific quality, data derived from both GLP and non-GLP studies should be managed as a same level. Evaluation data are generally referred to GLP safety studies aligned guidelines, however non-GLP studies are sometimes out of these two regulatory considerations. In many cases, non-GLP studies have been done under new scientific aspects like molecular toxicological sciences for elucidating the toxicological mechanism. It is not an exaggeration to say that reality gap for right safety evaluation should be derived from GLP-supremacy and guideline-follower. To have a right decision for safety evaluation, a “case by case” approach should be employed under the diverse sciences including new science and technology introduction.

Future aspect of regulatory science

Effective safety evaluation should have a place in the assessment of human relevance with making clear the toxicological action mechanism. To keep this concept, new approaches such as a systems-biology based on molecular toxicology should be sufficiently employed for the approval processes.

CHEMICAL RISK ASSESSMENT AND MANAGEMENT IN CHINA: HISTORY AND PROGRESSES

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Learning from history and the experiences of developed countries, China has made remarkable progress on chemical risk assessment and management in recent years. Under the current legal system, governmental agencies have taken necessary steps to adopt science-based approaches in their regulatory procedures. Several law and regulations were amended or revised to include risk assessment in the regulatory process, and new guidance have been promulgated to meet the countries' goal of globalization. This will present a historical perspective and recent progresses of chemical safety evaluation, risk assessment and management in China, and introductory overview of the newly revised regulations including the "China REACH" (MEP Order No 7) on Environmental Management of New Chemical, the "China GHS" (Decree 591) on Dangerous Chemical Control, "translated" SFDA Guidance from ICH, and to be revised regulations and guidance on pesticide. This presentation will also provide a comparison with US and other international standards in regulations, procedures and guidelines, address other important issues in regulatory toxicology such as GLP, animal welfare, and discuss the challenges and opportunities with personal experience both in the US and China.

METABOLIC DYSREGULATION AND ITS CLINICAL IMPLICATION IN OVARIAN CANCER

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Aerobic glycolysis, so called Warburg effect, is known as a hallmark of cancer. Cancer cells inevitably take advantage of glycolysis to meet the increased metabolic demand: rapid energy generation and macromolecular synthesis. It has been suggested that increased activity of hexokinase II (HKII), a rate-limiting enzyme of glycolysis, especially voltage-dependent anion channel (VDAC)-binding forms of HKII might be important for cancer cells to evade apoptosis. Avoidance of apoptosis can lead to a resistance to anti-cancer treatments such as chemotherapy. The prognosis of ovarian cancer remains poor mainly due to chemoresistance. The clinical usefulness of positron emission tomography (PET), which was invented using the Warburg effect in cancer cells, suggests that metabolic alterations could have a crucial role in cancer development. Therefore, the manipulation of the metabolic derangement could be an effective strategy to overcome the chemoresistance in ovarian cancer. We will discuss the clinical implication of metabolic dysregulation in ovarian cancer in terms of the association between increased glycolysis and chemoresistance.

NATURAL COMPOUNDS AS MODULATORS OF INTRACELLULAR SIGNALLING PATHWAYS AND OXIDATIVE STRESS IN MAMMALIAN CELLS AND *CAENORHABDITIS ELEGANS*

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Natural compounds e.g. from plants, marine organisms or fungi are a rich source of chemically unique secondary metabolites. The pharmacological potential of these compounds is increasingly recognized. We investigated toxicological aspects of various compounds to identify lead structures responsible for the biological activity. Therefore we investigated the toxicity of the substances (apoptosis/necrosis), effects of intracellular signalling pathways (e.g. Nrf2, FOXO, NFkB, MAPK) and oxidative stress in different mammalian cell lines. Distinct compounds were also analyzed using the model organism *Caenorhabditis elegans*. Several natural compounds, e.g. kahalalides show strong toxicity in distinct cell lines inducing apoptotic cell death. Pterocarpanes isolated from *Erythrina addisoniae* showed strong toxic effects via inhibition of MAPK signalling. Distinct flavonoids modulate Nrf-2 signalling pathway as well as NFkB signalling in various cancer cell lines. The effects of several flavonoids were also investigated *in vivo* using the model organism *Caenorhabditis elegans*: Distinct substances modulated Nrf2 (SKN1) and FOXO (DAF16) signalling, decreased the formation of reactive oxygen species and mediate an increase in stress resistance.

In conclusion, analysis of natural compounds isolated from different sources revealed that distinct substances exhibit prominent biological activities against various cancer cell lines by modulation of intracellular signalling pathways and oxidative stress. Several substances also mediate stress resistance in *Caenorhabditis elegans* accompanied by modulation of signalling pathways.

SELECTED WEST AFRICAN PHYTOCHEMICALS AND CHEMOPREVENTION

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Compelling epidemiological and experimental findings evidences indicate that plant-based diet rich in a wide variety of fruits and vegetables are effective in preventing life threatening diseases. Thus the search for novel chemopreventive agents acting on specific and/or multiple molecular and cellular targets hold promise as a rational strategy to the control of diseases including cancer. West Africa abounds in plants useful for various chemotherapeutic purposes Thus phytochemicals such as saponins and alkaloids, terpenes, steroids, coumarins, flavonoids, phenolic acids, lignans, xanthones, anthraquinones, edotides and sesquiterpenes have been isolated from *Vernonia amygdalina* (bitter leaf) a medicinal plant in West Africa. Epivernodalol characterized from *Vernonia amygdalina* using spectroscopic methods including ^{1}H-NMR, ^{13}C-NMR, MS, UV and IR spectra exhibited significant antioxidant properties in various model systems, induced phase 2 antioxidant enzymes and was active against skin cancer as demonstrated by its efficacy in HT-144 skin melanoma cell line. Kolaviron, a natural antioxidant biflavonoid isolated from the seeds of *Garcinia* kola (Guttiferae) indigenous to West Africa elicited striking inhibitory effects on diverse biochemical and molecular events associated with the multistage process of carcinogenesis. Specifically, kolaviron up-regulated antioxidant defense capacity, modulated gene expression, signal transduction mechanisms and reduced *in vivo* markers of oxidative damage to lipids, proteins and DNA. Furthermore, kolaviron suppressed Dimethylnitrosamine-induced oxidative damage and expression of cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) by inhibiting nuclear factor kappa B (NF-κB) and activator protein-1 (AP-1) in rat liver. Kolaviron elicited antiproliferative properties by inhibiting the growth and survival of both colon adenoma (LT97) and carcinoma cells (HT29). Kolaviron inhibited the induction of stress-inducible proteins clusterin and heat shock proteins apoptosis-related proteins, caspase-3 and caspase-9, Fas and Fas-L induced by ethylene glycol monoethyl ether in rat's testes. Kolaviron prevented Atrazine-induced changes in the expressions of p53, Bax, Bcl-2, p21, and mRNA levels of caspase-3 and caspase-9 in SH-SY5Y human neuroblastoma cell line. These phytochemicals exert chemopreventive effects by modulating intracellular signaling cascades, stress response and apoptotic proteins and are therefore promising candidates as prophylactic agents in chemoprevention.

Keywords: Kolaviron, Epivernodalol, *Garcinia* kola, *Vernonia amygdalina*, Chemoprevention, Apoptosis, Signaling

SAXITOXINS IN FRESH WATERS

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Toxic cyanobacterial blooms in lakes and reservoirs have been reported all over the world, and they are often dominated by hepatotoxins, i.e. microcystins (MCs), nodularins (NOD) and cylindrospermopsin (CYN), and sometimes by neurotoxins, i.e. anatoxin-a (ANA) and saxitoxins (STXs). The STXs are produced by bloom-forming microalgae (mainly marine dinoflagellates). These compounds are neurotoxins (more specifically, potent voltage-gated sodium channel antagonists) that can cause numbness, paralysis and death in mammals via respiratory arrest. STXs are also produced by freshwater cyanobacteria; therefore, there is a potential for these toxins to be transferred through the freshwater food web to pose a risk to human consumers of freshwater products contaminated by STXs. In Brazil, STXs and ANA are produced in freshwater systems by cyanobacterial genera *Anabaena*, *Aphanizomenon*, *Planktothrix* and *Lynbya*. In contrast, cases reported in Europe and the USA, *Cylindrospermopsis* species are the most common genera that biosynthesize STXs. The accumulation of saxitoxins (STXs) in fish from freshwater aquaculture was investigated for the first time in the present study. Cyanotoxins have been monitored in liver and muscle samples of *Oreochromis niloticus* by chromatographic methods, both before and after the depuration process. The results show that tilapia can accumulate STXs. Our findings suggest that depuration with clean water is an alternative process to eliminate STXs from fish and, therefore, improve the safety of tilapia for consumers.

RECENT REVIEW OF MARINE TOXIN POISONING IN TAIWAN

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The causative agents of seafood poisonings in Taiwan were tetrodotoxin (TTX), paralytic shellfish poisons (PSP), grass carp bile toxins, ciguateric toxins, excess dose of vitamin A, histamine, pyropheophorbide, and dinogunellin. Among them, TTX, ciguateric toxins, PSP and excess dose of vitamin recently caused the food poisoning and are concerned problems. The causative marine animals were identified as puffer, processed puffer products, octopus, goby, and some kinds of gastropods. Furthermore, TTX-containing animals in Taiwan were found to include puffer, octopus, goby, xanthid crab, gastropod, starfish, and flatworm. Those dried dressed fish fillets have caused some food poisoning incidents and been proved due to adulteration of toxic puffers. Among those cases, SDS-PAGE, IEF and PCR methods for identifying species of puffers and their products have been developed. PSPs were identified as the causative agent of several shellfish poisonings and have studied to distribute in the purple clam, xanthid crabs, and gastropods. The source of PSP was the toxic alga *Alexandrium minutum,* which appears in the winter period (December to March) in the inland aquaculture ponds in Taiwan. The toxin production of alga was affected by a variety of nutritional, environmental, and physiological factors. Most shellfish possessed high resistance to PSP, but the susceptibility of shellfish to the toxic alga was quite different depending on species. Meanwhile, fish liver of several kinds of grouper recently induced hypervitaminosis A disease. It was found that the level of vitamin A increased with increasing the body weight and liver weight. These fish species were identified as *Etelis coruscans* and *E. carbunculus* by using SDS-PAGE and PCR methods. Following this way, ciguateric fish *Lutjanus bohar*, *Masturus lanceolatus* and *Gymnothorax javanicus* were also identified as causative fishes of food poisonings. Other marine toxins including diarrheic shellfish poisons (DSP), neurotoxic shellfish poisons (NSP), and amnesic shellfish poisons (ASP) are no poisonings in Taiwan.

FOOD-DERIVED FURAN EXPOSURE, MECHANISMS OF CARCINOGENIC ACTION AND RISK ASSESSMENT

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Furan is found in various food and beverages such as coffee subjected to high temperature during preparation. Several studies have shown that furan is a hepatocarcinogen in rats and mice but the mechanisms, dose response relationship and relevance to human dietary intake is still uncertain. There is a relatively low margin of exposure between estimated human exposure and doses that cause a high tumor incidence in rodents. There is some evidence of genetic toxicity of furan at concentrations close to toxicity and of a major metabolite cis-2-butene-1,4-dial. In addition, oral doses as low as 2.0 mg/kg per day cause a proliferative response in specific lobes of the rat liver. A reversible change in expression of cell cycle and apoptosis-related genes was measured at 14 days but no alteration of the methylation status of the regulatory region of a range of genes analysed could be found, nor was there a change in expression of DNA-damage response genes. A higher dose in rats (30mg/kg daily for 3 months) caused an initial extensive centrilobular necrosis and a subsequent sustained biliary cell proliferation and intestinal metaplasia even after a 1-month off dose period. This change was found to be associated with evidence of inflammation, DNA damage and alteration of DNA methylation. The overall evidence suggests that various responses contribute to the hepatic carcinogenicity of furan including direct and secondary genotoxicity, epigenetic changes and modulation of control of cell cycle and apoptosis. The relative contribution of these factors differ as the dose level increases thus suggesting a non-linear dose response relationship that needs to be considered in the risk assessment.

IMMUNOMODULATORY EFFECTS OF ETAHNOLIC EXTRACT OF *THYPHONIUM FLAGELLIFORME* (Lodd.) Blume ON CYCLOPHOSPHAMIDE−TREATED RATS

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Keladi tikus, a folklore name for *Thyphonium flagelliforme* (Lodd.) Blume, is a traditional medicine that used for cancer treatment in Indonesia and South East Asia. The present study aimed to examine the immunomodulatory effect of ethanolic extract of *Thyphonium flagelliforme* (ETF) in cyclophosphamide (CPA)-treated rats. To induce immunosuppression, 150 and 110 mg/kg of CPA were intraperitoneally injected at day 1 and day 4, respectively. Simultaneously, ETF were orally administered once daily for 7 days with doses 250, 500, and 1000 mg/kg. The immunomodulatory effect were determined lymphocytes proliferation and phagocitosis macrophages activity were determined. Changes in plasma cytokines of tumor necrosis factor (TNF)-α, interleukin (IL)-1α, and IL-10 production, and numbers of CD8+ were also monitored in CPA-treated rats. The results showed that administration of CPA with a dose of 150 and 110 mg/kg in rats significantly suppress lymphocyte proliferation, phagocytic activity, TNF-α and IL-1α, and CD8 + cells. Administration of ETF (250−1000 mg/kg) reduced immunosupessive effect on lymphocyte proliferation, increase the number and activity of macrophages phagocytic in CPA-treated rats. The results demonstrated that CPA significantly supressed CD8+. The administration of ETF (250−1000 mg/kg) significantly improve the immunosupressive effect of CD8-induced by CPA.The study of plasma cytokine levels, administration of ETF (250−1000 mg/kg) also significantly reduce the suppressive effect of CPA on cytokine levels. The results indicated that the optimum dose of immunomodulatory properties is 250 mg/kg in CPA-treated rats. All taken together, the results conclude that ETF have immunomodulatory properties in CPA treated rat. This results suggest that ETF can reduce the side effect of chemotherapy especially immunosupressive effect that can be used as co-chemotherapy for cancer treatment.

HISTOPATHOLOGICAL AND BIOCHEMICAL CHANGES INDUCED IN RABBITS BY PROLONGED ORAL CYANIDE INTOXICATION

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Cyanide is widely distributed in the ecosystem and has been associated with goiter, disturbances of thyroid and CNS pathology. However, little information is available in the literature concerning toxic effects of cyanide on liver and kidneys. Hence, the objective of the present study was to determine the deleteriouseffects of prolongedoral cyanide insult on liver and kidney tissues, and associated biochemical changes in rabbits. For this purpose,12 locally bred adult male rabbits were allocated into two groups of 6 viz. control and experimental. Rabbits incontrol group were offered feed only while the rabbits inexperimental group received feed plus potassium cyanide (KCN) at 3 mg/kg body weight orally for a period of 40 days. Results demonstrated significantly increased (<0.05) serum activities of alanine transaminase, aspartate transaminase, alkaline phosphatase and lactate dehydrogenase enzymes in experimental group rabbits compared to control group. Likewise, the serum concentrations of urea, uric acid and creatinin were also significantly increased (<0.05) in experimental group rabbits compared to control group. None of the rabbit in both the groups demonstrated any of the gross changes in any organ on postmortem examination. Liver was normal in size, shape, texture and color. Kidneys were also normal in size and color. Histopathological examination revealed severe hepatocyte vacuolation and degeneration in liver of rabbits in experimental group. There was also excessive congestion and bile duct hyperplasia in experimental group rabbits liver. Kidneys of rabbits in experimental group demonstrated severe glomerular and tubular necrosis and congestion. In the tubular epithelial cells, pyknotic nuclei were also present.In conclusion,prolonged oralcyanide administration could have harmful effects on liver and kidney functions.

DEVELOPMENT OF AH (DIOXIN) RECEPTOR-BASED APPROACHES FOR THE STUDY OF TOXICOLOGY AND HEALTH EFFECTS OF DIOXINS AND RELATED CHEMICALS

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Exposure to and bioaccumulation of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD, dioxin) and related compounds can produce a wide variety of species- and tissue-specific toxic and biological effects, including tumor promotion, lethality, birth defects, hepato-, neuro-, and immunotoxicity, endocrine disruption, dermal toxicity. Proper epidemiological, risk assessment and exposure analysis of dioxins requires accurate measurements of these chemicals both in the species of interest and in various exposure matrices. Currently, besides the high-resolution instrumental analysis method as the “gold standard”, the numerous bioanalytical methods have also been developed and used for the detection of these chemicals and also used for the related toxicological studies, the majority of which take advantage of the ability of these chemicals to activate the aromatic hydrocarbon receptor (AhR) and the AhR signal transduction pathway. The most sensitive and widely used bio-analytical approaches are cell-based reporter gene bioassay systems, including CALUX (Chemically Activated Luciferase Expression) and CAFLUX (Chemically Activated Fluorescent Expression) bioassays. These bioassays utilize recombinant cell lines containing stably transfected dioxin (AhR)- responsive firefly luciferase or enhanced green fluorescent protein (EGFP) reporter genes, respectively. The application of the bioassays has significantly promoted the basic research on dioxin induced toxic and health effects. While the current bioassays are very sensitive, increasing their lower limit of sensitivity, magnitude of response and dynamic range for chemical detection would significantly increase their utility, particularly for the exposure situation containing low levels of dioxins.

A COMPARISON STUDY OF SINGLE AND COMBINED CYTOTOXIC EFFECTS OF FUMONISIN B1, AFLATOXIN B1 AND OCHRATOXIN A ON HUMAN MONONUCLEAR BLOOD CELLS USING METHYL TETRAZOLIUM ASSAY, COMET ASSAY AND FLOW CYTOMETRY

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Simultaneous exposure of human to different mycotoxins at low and/or high doses might have additive or synergistic effects on consumers' health. This study investigated the single and combined effects of fumonisin B1 (FB1) and ochratoxin A (OTA), and aflatoxin B1 on mononuclear cells obtained from healthy human. The MTT assay test showed an inhibition of cell viability over time and concentration. While the assessment of the cell DNA damage by Comet assay technique and Flow Cytometryfor apoptosis and DNA cleavage induction confirmed the results previously obtained on MTT assay that the effects of the studied mycotoxins in combination showed synergistic cytotoxic effects as compared to the effect of single mycotoxin and this increased over time and concentration of exposure. Additive to synergistic effects were observed at high dose (40) suggesting that high doses or chronic exposure for low doses were able to induce synergism. In addition, it was also shown that FB1 induced slowly its cytotoxicity, toxic and/ or inhibitive effect, as compared to AFB1 and OTA when exposed singularly to mononuclear cells. Finally a correlation study done on the results obtained from the three techniques revealed that all of technique positively correlated and in addition it showed that from one study, prediction of results for another technique could have been done. in conclusion, the exposure to to several mycotoxins simultaneously can induce different symptom and effect on the immune system.

RISK EVALUATION OF EXPOSURE TO LOW CONCENTRATION OF CARCINOGENIC MYCOTOXINS – MOLECULAR EVIDENCE OF SYNERGYSTIC EFFECTS

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Crops are susceptible to fungal attack in field or during storage. These fungi may produce mycotoxins which are very stable. We have analysed several cereals (rice, wheat, maize) but also olives; coffee; spice, from different origin (Vietnam, Thailand, France, Moldavia, Czech Republic, Morocco). The contamination of rice and spice was at alarming rate; especially AFB1. OTA, AFB, CIT and FB were also detected in maize and wheat. All samples of ground coffee contain OTA ranging from trace (< LOQ, 5 samples) to 11.9 µg/kg. The amount of OTA passing in the beverage ranged between 20-140%. Based on a typical menu including some of these ingredients and using the average mycotoxin's amount for calculation, we observed that the tolerable daily intake (TDI) was respectively 39-fold; 7-fold and 3 fold higher than the virtual safety dose (VSD) established for AFB1, OTA and FB. The simultaneous presence of OTA with either CIT or FB or ZEA, modify human kidney cells (HK2) cell viability. The main covalent OTA DNA-adduct, found in human tumours, identified as C8 dG-OTA was increased by simultaneous presence of OTA with CIT/ZEA/FB. The synergistic effects are due to increase of ERKs and COX_2; and modulation of biotransforming enzymes. In the same way, in *in vivo* studies on rat and pig fed simultaneously by OTA and FB or CIT in feed, the formation of OTA specific DNA adducts including C-C8dG OTA adduct and the both OTHQ related adduct increased. The data indicate clearly that exposure to low concentration of mycotoxin which is considered as safe when they are present together can lead to dramatic effect. Until now, regulation does not take into account co-contamination and it is urgent to develop decontaminating process.

Keywords*:* Mycotoxin*,* risk evaluation, genotoxicity, OTA DNA adduct

RISK BASED APPROACHES TO ENSURE SAFETY OF COSMETICS: PREVENTION OF SKIN SENSITISATION

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Some low molecular weight chemicals when exposed to the skin can cause skin sensitisation, and upon re-exposure Allergic contact dermatitis can develop in such sensitised individuals. This is a permanent immunological change which affects a small but significant proportion of the population. Exposure to such chemicals (skin sensitisers) comes from multiple sources including cosmetic products that are frequently implicated in the clinical literature as causing allergic contact dermatitis. For this reason, when considering the introduction of new materials into cosmetic products a careful process of risk assessment for skin sensitization (consideration of hazard profile and consumer exposure) must be followed as part of the overall safety assessment. Over the past decade advances in our ability to characterize skin sensitisation hazard (local lymph node assay) and in our understanding of how consumers use cosmetics has enabled the development of a quantitative risk assessment (QRA) process for skin sensitization. The QRA incorporates information on skin sensitisation potency, adjusts for uncertainty in extrapolating from an experimental test system to a population using a cosmetic product via application of safety assessment factors and compares this to a consumer exposure. The QRA can be used as part of a weight of evidence considering other information, such as clinical data, allowing a decision on acceptability of risk of inducing skin sensitization. We present the QRA and its application to cosmetics products. Depending upon risk assessment outcome, additional risk management should be considered to demonstrate acceptability of risk. Here we present a case study pertaining to use of poly hexamethylene biguanide (PHMB) in an underarm product: where clinical epidemiology data was generated both prior to and after a change in exposure to demonstrate the acceptability of the risk of induction of sensitisation following an increased exposure to PHMB.

TAURINE AN EARLY BIOMARKER OF DRUG-INDUCED LIVER NECROSIS

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Drug-induced hepatotoxicity or liver injury is one of the most important causes of liver diseases occurs in 1/1000 to 1/10000 of patients who receive therapeutic doses of various drugs. More than 50% of secondary acute liver deficiency is caused by drugs which acetaminophen is the most common. Taurine is one of the most abundant free amino acids in the body which does not enter proteins' structures. Taurine has already been studied as a biomarker in acetaminophen overdose, carbon tetrachloride poisoning, muscular necrosis, myocardial infracts, stroke, and many other clinical disorders. This fact encouraged us to suggest taurine as a non-specific biomarker of acute liver necrosis caused by hepatotoxic agents. Thirty patients poisoned with hepatotoxic agents were recruited to the study in Sina Hospital of Tabriz. Five millilitre blood samples, four times, at admission, 12, 24, and 48 hours following overdose were collected from their brachial vein. Plasma taurine concentration was determined using an already developed HPLC method with florescence detection. Liver function test (LFTs) was routinely performed in the biochemistry laboratory of Sina Hoapital. A non-parametric student t-test (Mann-Whitney) showed that the plasma concentration of taurine was increased at the first 12 hours following overdose compared to healthy controls ($P<0.0001$). According to our and previous studies, unlike other biomarkers of liver necrosis which are increased after 48 hours following overdose of a hepatotoxic agent, plasma concentration of taurine is raised significantly in the first 12 hours. Therefore, introducing taurine as a non-specific biomarker could be an important progress in the diagnosis and prognosis of liver necrosis. Plasma taurine concentration is relatively stable, easy to measure and non-elevated in a transient manner. However, further studies with large numbers of patients in a long period of hospitalization are needed.

Keywords: drug-induced hepatotoxicity, liver injury, Tabriz, Iran, Sina, taurine

UNDERSTANDING THE MECHANISM OF LAPATINIB-INDUCED HEPATOTOXICITY: AN IN VITRO-IN VIVO INVESTIGATION

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The use of molecular targeted therapy circumvented the dreaded side effects of conventional chemotherapy such as alopecia, gut irritation, nausea/vomiting and immunosuppression. Unfortunately, a different kind of toxicological outcome has marred the therapeutic success of these agents: where hepatotoxicities, hand and foot syndrome, neutropenia and cardiotoxicities are some of the major pathologies reported with the newer agents in the market. Here, we attempt to understand the mechanism of toxicity of a keynote EGFR/HER2 inhibitor, lapatinib, in order to derive plausible recommendations for the clinical management of such adverse events. Through incubation of either lapatinib or its dealkylated metabolite with human liver microsomes in the presence of glutathione, we observed the formation of a reactive metabolite-glutathione adduct derived from the O-dealkylated metabolite of lapatinib. Metabolite reactivity was also demonstrated with the mechanism-based inactivation of CYP3A4, supporting a clinical relevance in drug-drug interactions. In a pilot retrospective study involving 120 patients, those receiving the combination of lapatinib and the P450 inducer dexamethasone were 4.57 times more likely to develop hepatotoxicity and 3.48 times more likely to develop a clinically important change in alanine aminotransferase than compared to the other group. This clinical finding was reproduced in a metabolically-competent liver cell line, TAMH, whereby the administration of dexamethasone led to an enhanced cytotoxicity of lapatinib. This outcome supports the hypothesis that a metabolite of lapatinib could be accountable for the observed toxicity. Overall, we confirmed that lapatinib can be metabolized to chemically reactive species with the potential to lead to subsequent toxicities via direct binding to critical targets, or indirectly through inhibition of P450 enzymes, leading to drug-drug interactions. The dichotomy of these effects will be more aggressively determined in larger scale clinical studies.

STUDY OF INCIDENCE OF AMPHOTERICIN B SIDE EFFECTS AND NEPHROTOXICITY RISK FACTORS IN PATIENTS UNDER CHEMOTHERAPY

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Drugs used for treatment of cancer have cytotoxic effects with an inhibitory effect on bone marrow. This is more true for therapeutic agents used in hemopoietic cancers treatment. The drug regimens used to treat these types of cancer includes several medicines with simultaneous inhibitory effects on bone marrow. The consequences of this inhibition, is a decrease in production of defence cells and therefore reduced body's ability to fight pathogens. Presence of infection in the body is usually associated with fever and the fever due to bone marrow suppression is called febrile neutropenia. One of the most important drugs in treatment of febrile neutropenia is amphotericin B. This medicine has some immediate side effects such as: hypothermia, hyperthermia, hypotension, hypertension, nausea, diarrhoea, muscle and joint pain or irritability. There are also a series of side effects related to its toxicity of this drug such as: liver disorders, peripheral neuropathy, nephrotoxicity, anaemia and increased renal potassium excretion that causes hypokalaemia and hypomagnesaemia. The aim of the present study was to examine prevalence of side effects amphotericin B in patients with neutropenia fever. In this study, 100 hospitalized patients who were receiving amphotericin B were enrolled to the study. A questionnaire was designed and completed by the information in patients records and direct questions from patients. The data was analysed using SPSS.The results of this study showed that a total of 44% of the subjects were female. Their mean age was 38.6 ±16.5 years old. The cause of hospitalization was ALL (74%), AML (18%) and NHL (8%). Amphotericin dosage was 211.2 ± 130.1 mg (25-600 mg). 100 patients treated with amphotericin B, suffered a series of side effects. The most common side effects included: fever (83%), headache (79%), myalgia (60%), dizziness (57%), restlessness (54%), jointpain (52%), diarrhoea (46%).With increase in cumulative dose of amphotericin B, rises in serum creatinine and urea concentration happened which was an indication of nephrotoxicity. Treatment duration and cumulative dose of amphotericin B had no significant effects on serum potassium and sodium concentrations and blood cellcount. One of the most important side effects of amphotericin B that limits its usage is nephrotoxicity. There are several methods that can be employed to reduce nephrotoxicity of amphotericin B. Liposomal formulation of amphotericin B has the lowest nephrotoxicity. In Iran non-lipid form of amphotericin is used routinely and therefore it is recommended to use other formulations. There are also other methods that can be employed for reducing amphotericin B nephrotoxicity. Examples of such methods are slow infusion rate and administration sodium, potassium and magnesium salts prior to amphotericin B administration.

HORMONAL DISTURBANCES CAUSED BY BENZENE IN RAT

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Benzene is a common industrial solvent. Various industries use benzene to make chemicals viz.: styrene and cyclohexane. It is also used in the manufacture of dyes, detergents and pesticides. The most common exposure to benzene occurs through autoexhaust, industrial emissions and cigarette smoke. Strong experimental evidence suggests that benzene metabolism plays a critical role in its toxic manifestations. The carcinogenic and myelotoxic effects of benzene have been associated with oxidative stress and related mechanisms. However, effects of benzene exposure on endocrinal disturbances are not known. Present study describes the effects of benzene on a few selected hormones. Hormonal assays for T3, T4, ACTH, insulin, testosterone, progesterone and estradial were made using ELISA kits. Duration and dose dependent effects were observed on there hormones. Adaptive response was recorded for T3, T4, insulin and sex hormones after 45 and 60 days of exposure to benzene. Thus these toxicological responses considered within a time and dose response continuum suggest that adaptation at molecular level occurs after benzene exposure. Alterations of pathways and networks at the gene, protein or metabolic level of organization need to be determined. However, present study suggests that benzene is a potent endocrinal toxin as well.

INCREASED BLOOD HEME OXYGENASE-1 LEVELS IN HIGH SILICA EXPOSURE NON-SILICOSIS STONE MILL WORKERS

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Heme oxygenase-1 (HO-1) is a lung inflammatory marker that related to silica exposure in rat model and patients with Silicosis disease. In the previous studies, this marker is proposed as a sensitive indicator of silica exposure which will be advantage for health surveillance in silica exposed worker. The aim of this study was to determine a relation between HO-1 and silica exposure levels in stone mill workers with no Silicosis disease. The subjects were 102 workers from Northern part of Thailand. Lung pathology was assessed by using chest x-ray and pulmonary function test was assessed by spirometer. Ambient silica was quantified by standard method. Serum HO-1 was measured by Sandwich Enzyme Immunoassay. The results of this study showed that the ambient silica level mean was 10.70 mg/m^3 which higher than the recommended level by Occupational Safety and Health Administration (OSHA) guideline (8-h TWA PEL:10 mg/m^3). The film x-ray showed no lung pathology in any worker. The silica exposure level was significant negatively related to a low lung capacity whereas HO-1 was positively related to increased silica levels. HO-1 also showed significant correlation to Forced Expiratory Volume in One Second and silica level. After adjustment by age, smoking status and working period, the level of HO-1 in the high silica exposed group (>10 mg/m^3) was significantly 2 times higher than the level of HO-1 in the low silica exposed group (<2 mg/m^3) (209.96 vs 428.32 ng/ml). Therefore, the increasing of HO-1 level was independently from age, smoking status and working period. These results were supported the assumption of the relation between HO-1 with pulmonary function and HO-1 with silica exposure in the non-silicosis subject. This marker should be further developed for use as an indicator of lung pathology which caused by silica exposure.

Keywords: Silica, Heme Oxygenase, Stone mill, Lung function

HEAVY METALS IN PIG PRODUCTION : A CONCERN FOR PUBLIC HEALTH

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Consumption of pork products is traditionally preferred in most of Asian countries, and has increased in the last 10 years as a result of rising incomes and urbanization. The safety of edible animal tissues for human consumption is a priority for public health. Some of the contaminants that may be found in pork products come from the pig diets. Heavy metals (mainly cadmium, lead, arsenic) in animal feeds can originate either from contaminated feedstuffs or from supplemental sources of additives, especially essential trace minerals like zinc, copper, iron or manganese. When contaminated diets are fed to pigs, heavy metals accumulate preferentially in the target organs whereas concentrations in muscle remain low. Cadmium (Cd) is accumulated in the kidney at a higher rate than in the liver. Recent analytical surveys showed that one fourth of pig kidneys in Thailand was above the regulatory limits for human consumption due to an excess in Cd. The percentage of unsafe products could not be only explained by potentially Cd polluted areas. Thus, it was hypothesized that Cd in pig feeds could come from contaminated sources of dietary trace minerals. Recent analysis on batches of zinc oxide products utilized in animal nutrition showed high levels of contaminants. Supplementation of weaning pig diets with zinc oxide *at pharmacological levels*, about 25 times more than the nutritional requirements, is common to secure growth performance and/or reduce post weaning diarrhea of the piglets. Although the period of supplementation is early in the pig life, the long biological half-life of cadmium causes a risk of high Cd levels in the kidneys at slaughtering. A stricter Quality Control system in the pig production chain and regulatory measures could enhance the food safety.

QUANTIFICATION OF PESTICIDE USE IN EGYPT

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The use of pesticides in Egypt has a very long history. Ash and some naturally occurring matters were used in ancient Egypt to control stored grain and other pests. In modern years, the organized use of pesticides in Egypt started with the introduction of cotton to the Egyptian fields in the 1940s. Following introduction of the chlorinated hydrocarbon pesticides, the use of pesticides in Egypt has increased gradually until reaching a level of 42,000 tons as formulated products, about 20,000 to 30,000 tons of active ingredients (a.i.) in the early 1960s. The Government as represented by its Ministry of Agriculture was mostly the solo applicator of agricultural pesticides until the market was privatized in nearly twenty five years ago. This privatization has lead to sharp decrease in the agricultural use of pesticides as farmers became responsible for baying their needs of these. During the years between 1985 and 2005, the annual use of pesticides declined to reach a rang fluctuating between 4,000 and 5,000 tons as estimated as a.i. From 2005 to end of 2011, the average annual use of agricultural pesticides in Egypt is 6,326 tons a.i. per year with insecticides, as a category, always being in the top followed by fungicides then comes herbicides. Egyptian farmers grow crops two or three times in one year on the same soil. The total cropping area in Egypt is about 6.3 million hectares (about 14 million feddan). It means that the average rate of pesticides use in Egypt is about 1.00 kg of a.i. per hectare. As there about 1,411 million hectares of arable land in the world and the world uses 2.427 million tons of pesticides (a.i.) per year, the average use of these materials in the world is 1.562 kg per hectare per year.Based on these calculations, the annual average use of agricultural pesticides in Egypt is only two thirds of that of the world. A suggestion to estimate risk from exposure to pesticides in a country is proposed in this study. Analyzing the collected data also indicates that all pesticides used in Egypt are similar to those approved by the European Commission and or registered by the US Environmental Protection Agency.

ARE THE NEW CHINESE CHEMICALS REGULATIONS CATCHING UP WITH REACH?

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International harmonization of regulatory systems for industrial chemicals contributes to more efficient protection against the hazards and promotes free trade. Since 2006, the regulation of chemicals has been thoroughly reformed in both the EU and China. This study investigates the differences and similarities between the EU regulations "Registrations, Evaluation, Authorization and Restriction of Chemicals" (REACH) (Regulation (EC) No 1907/2006) and "Classification and Labeling" (CLP) (Regulation (EC) No 1272/2008) and China's three recently updated regulations on both new and existing chemicals, namely "Measures on the Environmental Management of New Chemicals", "Regulations on Safety Management of Hazardous Chemicals" and China's GHS National Standards. The regulations are compared in terms of aim of legislation, coverage of substances, content of legislation, distribution of legal responsibilities among the actors, criteria of prioritization, data collection and generation, data sources, the system of classification and labeling, restrictions, authorization, and communication requirements. Although there are important differences between REACH and China's chemicals regulations, the similarities show that there are strong common trends in the development of industrial chemicals management.

CARBOXYDEXTRAN-COATED SUPERPARAMAGNETIC IRON OXIDE NANOPARTICLES AFFECT THE LONG-TERM FATE OF KUPFFER CELLS AND MACROPHAGES

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Nanotechnology and the medical application of nanoparticles are rapidly growing fields. Due to their small size, nanoparticles exhibit novel physical, chemical, and biological properties, which differ from those of corresponding bulk material. Despite their frequent industrial and medical applications, our knowledge about interactions of nanoparticles with living cells as well as their effects on cellular functions remains rather limited. Iron oxide-containing magnetic nanoparticles have been successfully used in magnetic resonance imaging (MRI) of liver tumors and atherosclerotic plaques as well as in hyperthermia and drug delivery. Upon intravenous injection of carboxydextran-coated superparamagnetic iron oxide nanoparticles (SPIO) for diagnostic purposes, such particles are rapidly incorporation into Kupffer cells, the hepatic macrophages. This phenomenon can also be studied in mice, where we found that such injections trigger delayed apoptosis and subsequent Kupffer cell depletion. Confocal microscopy of macrophages treated in vitro with SPIO revealed that the internalized SPIO are confined to lysosomal vesicles, where they colocalize with α-glucosidase. We show that both, macrophages as well as recombinant α-glucosidase degrade the carboxydextran shell of SPIO in a time-dependent manner. After uncoating, the unprotected iron oxide core triggers an increased production of radical oxygen species (ROS) leading to sustained activation of the c-Jun N-terminal kinase (JNK). The proinflammatory cytokine TNF-α increases the apoptosis rate, the ROS production and the JNK activation elicited by SPIO. Notably, the SPIO-induced apoptosis of Kupffer cells can be abolished by treatment of the mice with the radical scavenger edaravone. Thus, SPIO-based contrast agents are retained for extended time periods by hepatic macrophages, where they elicit delayed cell death that can be blocked by suitable radical scavengers. These data imply that the cytotoxic effects of iron oxide nanoparticles require more intensive in vivo studies, which should be considered for biomedical applications. Funded by the German Research Association (DFG).

INVOLVEMENT OF ROS GENERATION IN SILVER NANOPARTICLE-INDUCED A549 CELL TOXICITY

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Silver nanoparticles (AgNPs) are increasingly used in a large number of consumer products. Consequentially, the potential adverse effects associated with exposure to them are of concern. The present study investigated the mechanisms of AgNP (< 100 nm) toxicity in relation to the generation of reactive oxygen species (ROS) in A549 cells. It was found that AgNPs caused ROS formation in the cells, a reduction in their cell viability and mitochondrial membrane potential (MMP), an increase in the proportion of cells in the sub-G, S phase arrest and down-regulation of the cell cycle associated proliferating cell nuclear antigen (PCNA) protein, in a concentration- and time-dependent manner. Pretreatment of the A549 cells with N-acetyl-cysteine (NAC), a ROS scavenger, decreased the effects of AgNPs on the reduced cell viability, change in the MMP and proportion of cells in the sub-G1population, but had no effect on the AgNP-mediated S phase arrest or down-regulation of PCNA. This study proposes that the toxic effects of AgNPs in A549 cells are mediated via both ROS-dependent (cytotoxicity) and ROS-independent (cell cycle arrest) mechanisms.

GENOTOXIC EFFECTS IN VITRO OF EXPOSURE TO NANOPARTICLES WITH DIFFERENT PHYSICO-CHEMICAL PROPERTIES

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Genotoxic effects of nanoparticles (NPs) with different physico-chemical properties, namely: commercially available silica nanoparticles, Ludox CL (nominal size 21 nm, alumina coated) and CL-X (30 nm, sodium stabilized); titania from JRC: NM-102 (15-25 nm, anatase), NM-103 (20 nm, hydrophobic rutile, alumina coated) NM-104 (20 nm, hydrophilic rutile, alumina coated), NM-105 (22nm, 85% anatase/15% rutile); and zinc oxide JRC: NM-110 (42 nm, uncoated) was determined using comet assay in murine (3T3-L1) and human (WI-38) fibroblast cell lines as well as in epithelial cell lines; A549, BEAS-2B and Caco-2. In some cell lines after exposure for 24 hours to titania nanomaterials a statistically significant increase in DNA damage parameters in comet assay was observed. However, generally the effect was not common for all cell lines, and they did not show a clear concentration-effect relationship. In contrary, genotoxic effect of ZnO NPs was observed in all cell lines studied, starting from 10 μg/ml in the case of BEAS-2B cells. No genotoxicity was observed in 3T3-L1 fibroblasts after exposure to Ludox CL and CL-X nanoparticles. Part of this work was supported by EU projects: NanoInteract (NMP4-CT-2006-033231) and ECNIS^2 (7PR/2011/266198). Part of this communication arises from the Joint Action NANOGENOTOX which has received funding from the European Union, in the framework of the Health Programme. It reflects only the authors' views and the Executive Agency for Health and Consumers is not liable for any use that may be made of the information contained therein. The nanomaterials presented here come from the JRC repository and are also studied in the sponsorship program of OECD.

INNOVATIONS TOWARDS A NON TOXIC ENVIRONMENT AND HOLISTIC HEALTH IN DEVELOPING COUNTRIES AND BEYOND

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Holistic health is the health of the 21st century. In the past, optimistic market size expectations led to chemical storage capacity oversizing gas leaks in Bhopal. Town growth and larger than expected - impacted population were reported both in Bhopal ,India and and in Map Ta Put,Thailand. The consequences of these accidents extend beyond the tragic incidences. Remaining sites not properly removed, economic incentives as well as apathy from citizens create concerns even at decades after these events. Local community risk analysis and pre-cautionary risk management should be enhanced by all parties involved in chemical safety. Reduction of risk and uncertainties for risk reduction intervention effectiveness not only come from laboratory state of the art understanding of gene expression such as epigenetics but also come from real data collection using true scenarios and operational research of holistic health promotion following these tragic events aiming for complete recovery through stem cell differentiation and modulation of external and internal environment of the exposed citizens.Innovations through global networking and community outreach programs based on educational participatory learning by inquiry in high schools to create informed choices is also proposed as collaborative efforts among countries.

CATEGORY 1 : TOXICITY, CYTOTOXICITY AND GENOTOXICITY

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GENETIC BACKGROUND OF TESTICULAR GERM CELL TUMORS: FROM CHEMOSENSITIVITY TO CHEMORESISTANCE?

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Human testicular germ cell tumors (TGCTs) are histologically heterogenous neoplasms with variable malignant potential. Two main groups, seminomas and nonseminomas, differ biologically and clinically. Considered to have a common origin, little is known about their development and cellular mechanisms involved in response to therapy. In order to gain insight about the role of genetic background in development and clinical outcome of TGCTs, a set of genes involved in cell adhesion (*CDH1*, *APC*, *NME1)*, cell cycle regulation (*CDKN2A*, *RB1*, *TP53*), DNA damage repair (*BRCA1*, *MLH1*, *MSH2*, *RAD51*), apoptosis regulation (*BAX*) and multidrug resistance (*ABCG2*) was investigated in forty TGCTs. In TGCT tissue samples, occurrence of loss of heterozygosity (LOH) and microsatellite instability (MSI) were evaluated. Those genetic alterations provide information about whole genome integrity, as well as structural changes in particular key genes. Different patterns of LOH have been observed between the two TGCTs groups and no common structural genetic alteration was found, indicative of independent development for TGCT groups. LOHs of several genes with synergistic effect (*CDH1*, *CDKN2A*, *RB1*, *TP53*), as well as higher incidence of LOH in TGCTs harboring highly metastatic and chemoresistant histological components, may provide a clue to their clinical behavior. The analysis of genes involved in DNA damage repair showed no changes in agreement with the fact MSI has not been observed, and most of TGCTs respond well to therapy.

STUDY ON THE TOXICOKINETICS OF SCUTELLARIN IN BEAGLE

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Objective To identify the pharmacokinetic profile of scutellarin and the accumulation situation of its products, eighteen Beagle dogs were treated with drugs at three doses level for three months by intravenous injection administration. **Methods** During the long-term toxicity test of scutellarin injection on Beagle dogs, the blood was drawn at 0, 0.083, 0.25, 0.5, 0.75, 1, 2, 3, 4, 6, 8 and 24 h following treatment on the first day and the last day respectively, and then these samples were tested by API 4000 LC-MS/MS for drug blood concentration. **Results** The main pharmacokinetic parameters of first administration for high(100mg/kg), middle(50mg/kg) and low(25mg/kg) dose groups as follows: $AUC_{(0-t)}$ average were 56552.1ug/L×h, 19200.5ug/L×h and 2790.5ug/L×h, respectively; $AUC_{(0-\infty)}$ average were 56827.7ug/L×h, 19256.1ug/L×h and 2851.1ug/L×h, respectively; C_{max} average were 111620ug/L, 61296ug/L and 8813.67ug/L, respectively; $t_{1/2}$ average were 4.41h, 3.60h and 2.27h, respectively; $AUC_{(0-t)}$ average was positively correlated with administration dosage, and correlation coefficient was 1. The main pharmacokinetic parameters of last administration for high, middle and low dose groups as follows: $AUC_{(0-t)}$ average were 40956.7ug/L×h, 29394.5ug/L×h and 9492.4ug/L×h, respectively; $AUC_{(0-\infty)}$ average were 40959.5ug/L×h, 29718.7ug/L×h and 9898.2ug/L×h, respectively; C_{max} average were 106978ug/L, 84000ug/L and 30410ug/L, respectively; $t_{1/2}$ average were 0.46h, 3.34h and 11.77h, respectively. There didn't exist the linear relationship between $AUC_{(0-t)}$ average, C_{max} average and administration dosage. **Conclusion** Blood concentration of scutellarin in low dose group became higher obviously, and its systemic exposure concentration of low and high dose groups increased significantly. Therefore, it exists the risk of accumulation during long-term administration with scutellarin.

Keywords: Scutellarin; Toxicokinetics; API 4000 LC-MS/ MS

MUTAGENICITY EVALUATION OF THE HYDROCHLOROTHIAZIDE IMPURITY, 4-AMINO-6-CHLORO-1, 3-BENZENEDISULFONAMIDE, IN AMES TEST

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The presence of impurities in drugs may influence the efficacy and safety of pharmaceutical products even in very small amounts. Both pharmaceutical industries and regulatory authorities increase their focus on the subject, particularly considering that genotoxic impurities in pharmaceuticals at trace level may cause mutagenesis and carcinogenesis in humans. Hydrochlorothiazide is a widely used diuretic that is utilized singularly or in combination with other drugs for the treatment of hypertension. Hydrochlorothiazide contains certain impurities related to process/degradation and one of the impurities is 4-amino-6-chloro-1, 3-benzenedisulfonamide, which is mentioned by the US Pharmacopeia and European Pharmacopeia. Long-term use of the hydrochlorothiazide drug in hypertension patients also can bring along the chronic exposure to the impurity which has no report of the genotoxicity data. Taking into account the guidelines on the impurities, we aimed to investigate probable mutagenic effect of this impurity, in order to reach a conclusion where the data to be attained would cast light over the safety profile of the impurity. As an initial test in the evaluation of genotoxic effects of impurities, the mutagenicity determination was evaluated by the bacterial reverse mutation test (Ames test) using strains TA98 and TA100 of Salmonella typhimurium in the absence of metabolic activation system. A preliminary toxic dose range experiment was performed up to 5000 µg/plate and bacterial toxicity was observed at concentrations of $\geq$750 µg/plate. In the mutagenicity tests, when the revertant colony numbers of vehicle control plates were within the corresponding historical control data ranges and positive controls showed increased revertant colony numbers, there was no increase in the mean number of revertant colonies of doses 100, 200, 300, 400 and 500µg/plate in both tester strains. Results showed that the impurity, 4-amino-6-chloro-1,3-benzenedisulfonamide, was not mutagenic in the conditions performed.

INVESTIGATION OF THE TOXICITY OF EMISSIONS FROM WOOD AND ORIENTED STRAND BOARDS (OSB)

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Objectives: A human exposure trial was conducted to examine sensory and irritative effects of volatile organic compounds (VOC) emitting from pine wood and OSB. Moreover, cytotoxic and genotoxic effects of aerosols released from these materials and selected terpenes and aldehydes were investigated in A549 lung cells *in vitro*. **Methods:** Volunteers were examined for effects of exposure to emissions from pine wood (N=15), and OSB (N=24). Each subject was exposed under various exposure conditions in a 48 m³ test chamber. Chemophysical parameters were measured continuously. Sensory irritation, lung function and smell evaluation were recorded. The semantic differential (SD) was used to estimate odour perception. *In vitro*, cytotoxicity and genotoxicity (comet assay) of the emissions were recorded after 1 h of exposure of A549 cells at an air/liquid-interface (Vitrocell®). VOC were determined by GC/MS. **Results:** Except for a significant perception of odour, no evidence was found of adverse effects on eyes, nose, throat, upper airways or lung function after 2-h exposure up to levels of 13 mg/m³. Odour was rated as more "pleasant" than "unpleasant". Neither cytotoxic nor genotoxic effects were observed in vitro at total volatile organic compound (TVOC) concentrations up to 80 mg/m³. Terpenes did not induce toxic effects in A549 cells in concentration up to g/m^3; hexanal displayed cytotoxicity at 2,000 mg/m^3, and the α,β-unsaturated aldehydes 2-heptenal and 2-octenal predominantly emitted from OSB caused DNA-migration in concentrations of 100 and 40 mg/m^3, respectively. **Conclusions:** Exposure to high VOC concentrations released from pine wood or OSB did not elicit sensory irritation or pulmonary effects in healthy humans. VOC mixtures and terpenes did not induce effects in A549 lung cells. The lowest effect level of cyotoxicity and genotoxicity of some of the compounds was observed at much higher concentrations than those found in normal indoor air environments.

TOXICOLOGICAL CHARACTERIZATION OF VARIOUS METAL SULFATE MICROPARTICLES FOUND IN STACK EMISSIONS OF A COAL FIRED POWER STATION IN HUMAN LUNG CELLS A549 *IN VITRO*

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Objective: Ambient airborne particulate matter of varying size and composition is known to cause health effects in humans after inhalative exposure. In a recent study, the environmental impact of tire combustion was investigated in a coal-fired power station, and $ZnSO_4 \cdot H_2O$, $CaSO_4$ and $PbSO_4$ identified in the stack emissions. Based on these findings, we investigated these metal sulfate particles with regard to their physicochemical profile and toxic, especially genotoxic, potential in human alveolar epithelial cells (A549) in vitro. Moreover, we investigated their impact on signalling pathways. **Methods:** Cells were exposed to the various particles ($ZnSO_4 \cdot H2O$ as PM21; CaSO4 as PM6.7 and PbSO4 as PM2) under submerse conditions for 24 h. Transmission electron microscopy (TEM) and energy-dispersive X-ray spectroscopy (EDX) were used to examine cellular uptake of sulfate microparticles. ROS formation was investigated by dichlorodihydrofluorescin diacetate (DCFH-DA) assay and electron paramagnetic resonance spectroscopy (EPR).The DNA damaging effects were studied by the Comet assay and induction of micronuclei by the CB-MNvit test. **Results:** An increase in ROS induction was observed for all the sulfate particles analyzed, although to varying extent. $ZnSO_4 \cdot H_2O$ was the most active particle. The results of the genotoxicity assays revealed concentration-dependent DNA damage and micronuclei induction for $ZnSO_4 \cdot H_2O$ and $PbSO_4$, and to a lower extent for $CaSO_4$. It could further been shown that $ZnSO_4 \cdot H_2O$, but not $CaSO_4$ increased the NF-κB DNA binding activity and that this process was ROS-dependent since the ROS scavenger NAC could reduce the activation. Furthermore, $ZnSO_4 \cdot H_2O$ was able to activate JNK, a process also shown to be ROS-dependent. **Conclusions:** We were able to demonstrate that the sulfate microparticles analyzed caused several toxic effects after exposure to human lung cells in vitro. Since these metal sulfate particles may be globally abundant aerosols emitted into the atmosphere with the flue gas of coal-fired power stations, they could have considerable impact on the environment and health.

EVALUATION OF CONTACT SENSITIZING POTENTIAL OF FRAGRANCE MIX AND BALSAM OF PERU BY USING THE EX VIVO LLNA-BRDU METHOD

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Fragrance mix and balsam of peru are commonly used in cosmetic products. In the present study, *ex vivo* LLNA-BrdU method was used to evaluate the contact sensitizing potential of these cosmetic mixtures. Fragrance mix and balsam of peru at the concentrations of 0, 0.5, 5, 25, 50% in acetone:olive oil (4:1 v/v) (AOO) were applied topically on the dorsum of both ears of the 8-12-week-old female Balb/c mice. The stimulation index values and estimated concentration (EC3) values were calculated and potency classification was found for each mixture. According to the results of *ex vivo* LLNA-BrdU assays, EC3 values were found to be 3.09% (moderate) for balsam of peru and 4.44 % (moderate) for fragrance mix. Th1 cytokines (IL-2, IFN-γ) and Th2 cytokines (IL-4, IL-5) releases from lymph node cell culture as non-radioactive endpoints were also investigated. Cytokine analyses results indicate that both Th1 and Th2 cytokines are involved in the regulation of murine contact allergy and can be considered as useful endpoints.

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CYTOKINE PRODUCTION FROM THE CELLS IN THE THREE-DIMENSIONAL HUMAN SKIN MODEL CONSTRUCTED ON A COLLAGEN VITRIGEL MEMBRANE

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Introduction We previously reported cyokines release from a three-dimensional human skin model consisting of normal fibroblasts (NHSF), normal keratinocytes NHEK(F) and normal dendritic cells (NHDC) on a collagen vitrigel (VGC-KDF) by the treatment with skin sensitizer. However, which cells mainly relate to the cytokines production is unknown. In this study, we examimed cytokines release from each cell or the combination model of those cells treated with skin sensitizer. **Materials and Methods** NHSF or NHDC (matured or non-matured) or NHEK(F) seeded into 96 well plate and 24 hr incubated, and then the cells were exposed to DNCB or SDS for 24 hr. After 24 hr, viability of the cells were measured, and IL-8 and G-CSF were measured with ELISA. In another experiment, NHSF seeded on a collagen vitrigel (VGC-F), NHDC (non-matured) on a collagen vitrigel (VGC-D), both of NHSF and NHDC on a collagen vitrigel (VGC-DF), EPI-200 model and Labcyte-Epi-model were treated with same chemicals for 1 hr. After removal of these chemicals, these skin models were further incubated for 23 hr, and the amounts of cytokines in the supernatant were measured . **Results and Discussion** The order of IL-8 amount product from each cell was as follows:NHSF> NHDC(matured)> NHDC(non-matured)>NHEK(F) . Amount of IL-8 determined in VGC-KDF was highest and followed by VGC-DF> VGC-F> VGC-D. G-CSF was not detected in NHSF, NHDC, NHEK(F), EPI-200 model and Labcyte-Epi-model. These results suggested that G-CSFand IL-8 mainly produced from NHSF.

EVALUATION OF THE ESTROGENICITY OF DIFFERENT OILS USING IN VIVO ASSAYS IN RATS

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Evidence of estrogenic activity of a variety of oils was reported almost 40 years ago in a study which showed that mouse uterine weight significantly increased after dietary exposure to corn, peanut, olive, soybean, coconut, and rice oil. Very few and sporadic articles since then have supported the idea that certain oils may have estrogenic activity. Here we report the use of the Allen-Doisy and the uterotrophic assay in rats to study the estrogenicity of corn, almond, cod, coconut, and grapeseed oil. In the Allen-Doisy assay, estrogenic activity is evidenced by the induction of cornification of the vaginal epithelium in adult female ovarioctemized rats which was achieved by daily microscope observation of vaginal smears. Cornified cells are observed only on the estrous stage of the estrous cycle and are easily distinguished because of their size and shape. Animals were subcutaneously administered three consecutive doses of 300 µl of oil, one on the afternoon of the first administration day and in the morning and afternoon of the second day. Vaginal smears were observed for a period of ten days after end of administration. In the uterotrophic assay, estrogenicity was evaluated by the increase of uterine weight in rats at postnatal day 18, which were administered oil subcutaneously using the same dosing scheme described for the Allen-Doisy assay. On the fourth day, uteri were collected and weighed. Bisphenol A (20 mg/kg) and estradiol (50µg/kg) were used as positive control in both assays. Our data shows that both corn and almond oil have a strong and statistically significant estrogenic response in the assays although with potency several orders of magnitude lower than estradiol, while cod, coconut and grapeseed do not induce a response. The potential health impacts of estrogenic components in food oil deserve further attention.

Keywords: Estrogenicity, Oils, Uterotrophic assay, Allen-Doisy assay

TITANIUM DIOXIDE NANOPARTICLES-MEDIATED IN VITRO CYTOTOXICITY DOES NOT INDUCE HSP70 AND GRP78 EXPRESSION IN HUMAN BRONCHIAL EPITHELIAL A549 CELLS

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Titanium dioxide nanoparticles (TiO_2NPs) are increasingly being used in various industrial applications including the production of paper, plastics, cosmetics and paints. With the increasing number of nano-related products, the concern of governments and the general public about the health and environmental risks, especially with regard to occupational and other environmental exposure, are gradually increasing. However, there is insufficient knowledge about the actual affects upon human health and the environment, as well as a lack of suitable biomarkers for assessing TiO_2NP-induced cytotoxicity. Since the respiratory tract is likely to be the main exposure route of industrial workers to TiO_2NPs, we investigated the cytotoxicity of the anatase and rutile crystalline forms of TiO_2NPs in A549 cells, a human alveolar type II-like epithelial cell line. In addition, we evaluated the transcript and protein expression levels of two heat shock protein (HSP) members, Grp78 and Hsp70, to ascertain their suitability as biomarkers of TiO_2NP induced toxicity in the respiratory system. Ultrastructural observations confirmed the presence of TiO_2NPs inside cells. *In vitro* exposure of A549 cells to the anatase or rutile forms of TiO_2NPs led to cell death and induced intracellular ROS generation in a dose-dependent manner, as determined by the MTS and DCF assays, respectively. In contrast, the transcript and protein expression levels of Hsp70 and Grp78 did not change within the same TiO_2NPs dose range (25 – 500 µg/ml). Thus, whilst TiO_2NPs can cause cytotoxicity in A549 cells, and thus potentially in respiratory cells, Hsp70 and Grp78 are not suitable biomarkers for evaluating the acute toxicological effects of TiO_2NPs in the respiratory system.

EFFECT OF DIAZINON ON GDH GENE EXPRESSION AND INSULIN SECRETION IN RAT

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The main aims of the present study were to determine the effects of diazinon on gene expression of glutamate dehydrogenase (GDH) as the key enzyme of Langerhans islet for secretion of insulin and insulin secretion. Diazinon was administered intraperitoneally at doses of 15, 30, and 60 mg/kg. Langerhans islets were isolated from the pancreas of rats by a standard collagenase digestion, separation by centrifugation, and hand-picking technique. The activity and gene expression of the mitochondrial GDH was determined in the islets homogenates. Glutamate, C-peptide, and insulin were determined in plasma. Diazinon at all tested doses (15, 30, and 60 mg/kg) significantly ($p < 0.01$) decreased plasma insulin after 1 h while the values did not differ from control when examined after 18 h. Diazinon at all tested doses (15, 30, and 60 mg/kg) significantly ($p < 0.01$) increased concentration of C-peptide both 1 and 18 h post-administration. Diazinon at all tested doses (15, 30, and 60 mg/kg) significantly ($p < 0.05$) increased production of glutamate while the values did not differ from control when tested after 18 h. Administration of diazinon at doses of 30 and 60 mg/kg significantly ($p < 0.001$) increased activity of GDH after 1 h while all doses of diazinon increased GDH activity when measured after 18 h. Diazinon at dose of 60 mg/kg significantly ($p < 0.01$) decreased expression of GDH gene 18 h post-administration. It is concluded that GDH is a component of diazinon-induced changes in release of improper insulin.

Keywords: Insulin, Islets isolation, Glutamate dehydrogenase, Diazinon, Pancreas, Rat.

ASSESSMENT OF GENOTOXICITY AND CYTOTOXICITY OF DIOSCOREALIDE B IN HUMAN LYMPHOCYTES *IN VITRO*

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Dioscorealide B (DB) is an active compound isolated from *Dioscorea membranacea* Pierra (Hua-Kua-Yen in Thai) rhizomes. Huan-Kua-Yen is commonly used in Thai traditional recipes to treat bone and joint disease and cancer-related inflammation. Previous studies have demonstrated that DB was selectively cytotoxic to lung cancer cell (CORL-23) and breast cancer cell (MCF-7) but not keratinocyte cell (SVK). However, the genotoxic mechanism of DB was not yet determined especially in human lymphocytes. This study aims to investigate the genotoxic and cytotoxic activities of DB in human lymphocytes *in vitro* by sister chromatid exchange (SCE) assay. DB (dissolved in DMSO) was used to treat human lymphocytes at 10, 50, 100, 500, and 1000 µg/ml for 3 h. SCE was scored and analyzed. Plain RPMI and 0.4% (v/v) DMSO were used as negative controls whereas doxorubicin (0.1µg/ml) was used as the positive control. The results indicated that SCE levels induced by DB treatments (at all concentrations treated) were not significantly different from those of the negative controls. Niether mitotic index nor proliferative index was significantly different. Comparing to the crude ethanolic extract of *Dioscorea membranacea* rhizome (EED), the EED was cytotoxic at concentration $\geq$ 500 µg/ml as no mitotic cell was found. The usage of DB is, therefore, safe at concentrations of 10-1000 µg/ml. However, more *in vivo* studies are needed to clarify the proper dosage use and safety for patients.

This study was supported by Office of Higher Education Commission and Thammasat University, Thailand.

CHEMICAL CONSTITUENTS AND ANTIOXIDANT ACTIVITIES OF CLEISTOCALYX NERVOSUM FRUITS IN *IN VITRO* AND *IN VIVO* MODELS

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Cleistocalyx nervosum var. paniala, or Ma-kiang in Thai, is a purplish red fruit cultivated in northern Thailand. Its flesh is edible and is used to make juice, wine and jam. The previous study reported that the aqueous extract of C. nervosum pulp showed antioxidant activity and anticarcinogenesis. The present study investigated the fruit's chemical constituents and antioxidant activities, both in vitro and in vivo. Both aqueous and ethanol extracts (95% v/v) were used. The amount of polyphenols, flavonoids, cyanidin-3-glucoside and DPPH radical-scavenging capacity of the ethanolic extract were greater than those of the aqueous extract. The 95% ethanol extract of C. nervosum was further investigated for its safety and antioxidant activity in animal models. The administration of the ethanolic extract of C. nervosum at 5 g/kg bw was safe in wistar rats. Various concentrations of C. nervosum ethanolic extract at 100, 300 and 1000 mg/kg bw were intragavage fed to male wistar rats for 90 days. The ethanolic extract significantly decreased malondialdehyde (the end-product of lipid peroxidation) levels in liver, but not in serum. It also induced hepatic glutathione peroxidase activity. The results of this study showed that 95% ethanolic extract of C. nervosum contains a high content of anthocyanin that has antioxidant capacities both in in vitro and in vivo models.

Keywords: Cleistocalyx nervosum, Anthocyanin, Antioxidant activity

β-CATENIN INVOLVEMENT IN ARSENITE-INDUCED VEGF EXPRESSION IN NEUROBLASTOMA SH-SY5Y CELLS

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Arsenic contamination of the environment is widespread and especially evident in drinking water. Although it is a known carcinogen in humans, the mechanism by which arsenic induces carcinogenesis is not well understood. Among several effects of arsenic, arsenic-induced vascular endothelial growth factor (VEGF) expression has been suggested to play a critical role in arsenic-induced carcinogenesis. In the present study, we demonstrated that arsenite induced VEGF expression in neuroblastoma SH-SY5Y cells without induction of HIF-1α, a well-known transcriptional activator for VEGF suggesting that arsenite-induced VEGF expression in SH-SY5Y cells may not require HIF-1α activation. It has been reported that VEGF expression is regulated by multiple transcription factors including β-catenin. We therefore investigated whether β-catenin is involved in arsenite-induced VEGF expression in SH-SY5Y cells. Treatment of arsenite caused β-catenin accumulation in the nucleus. Additionally, arsenite treatment decreased activity of GSK3, an enzyme phosphorylates and targets β-catenin for degradation by proteasome without activation of its upstream kinase, Akt. Inhibition of PI3K/Akt which negatively regulates GSK3 activity by LY2940002 resulted in a decrease in arsenite-mediated β-catenin nuclear accumulation, and VEGF expression. These results suggested that β-catenin plays a role in arsenite-induced VEGF in SH-SY5Y cells, and the induction of β-catenin by arsenite is mediated by inhibition of GSK3 without activating its upstream kinase Akt.

Keywords: Arsenite, VEGF, β-catenin, HIF-1α, GSK3

ARSENIC ALTERS CHOLINERGIC SYSTEM AND DYSREGULATES INTRACELLULAR CALCIUM IN MICROVASCULAR ENDOTHELIAL HMEC-1 CELLS

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Our previous study has demonstrated that vascular endothelium played more significant roles than vascular smooth muscle in the arsenic-induced alteration in vascular response. We also reported that arsenic impaired the response of cholinoceptors to acetylcholine in the rat aortic ring. The present study was designed to investigate the potential mechanism underlying arsenic toxicity in the microvascular endothelial cells, HMEC-1. The MTT cell viability assay showed that arsenic (0.01-100 μM) caused concentration- and time-dependent HMEC-1 cell death which was further characterized as apoptosis by the Annexin-V/PI staining assay. The cytotoxic concentrations of arsenic (1-10 μM) increased the expression level of the cholinergic components including muscarinic acetylcholine receptor subtype M3 (M3 mAChR) and acetylcholinesterase (AChE), however the cholinoceptor antagonists including atropine (a nonselective mAChR antagonist), and mecamylamine (a nonselective nicotinic receptor antagonist) could not protect against arsenic-induced cell death. Arsenic also caused slow and sustained elevation of intracellular calcium levels ($[Ca^{2+}]_i$) and this effect correlated well with apoptosis. Furthermore, a specific IP_3 antagonist, 2-APB, attenuated $[Ca^{2+}]_i$, eNOS phosphorylation and apoptosis induced by arsenic. Altogether, these results suggest that arsenic induces an alteration of the endothelial cholinergic system and the dysregulation of the IP_3/$[Ca^{2+}]_i$,/eNOS signaling involved in arsenic-induced endothelial cell apoptosis.

EFECT OF ANTHOCYANIN-RICH EXTRACT FROM HUSK OF PURPLE GLUTINOUS RICE (*ORYZA SATIVA* VAR. *GLUTINOSA*) ON DIETHYLNITROSAMINE-INDUCED INITIATION STAGE OF RAT HEPATOCARCINOGENESIS

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Purple glutinous rice (*Oryza sativa* var. *glutinosa*) variety Kham Doisaket contains high amounts of polyphenols including anthocyanins as compared to other Thai rice. Several reports have presented that phytochemicals from inedible parts of plants can prevent cancer formation and reduce some degenerative diseases. This study aimed to investigate the safety, genotoxicity and antigenotoxicity of acidic methanol extract containing high anthocyanins of Kham Doisaket rice husk. Following the OECD Guideline 425 protocol to evaluate acute toxicity indicated the extract was nontoxic and its LD_{50} was greater than 2000 mg/kg bw. The clastogenicity and anticlastogenicity of the extract were studied using rat liver micronucleus assay. Male wistar rats were divided into 7 groups. Groups 1 to 5 were intragastrically fed with concentrations of rice husk extract varied from 100 to 1000 mg/kg bw for 28 days. Groups 6 and 7 were received distilled water as negative and positive control groups. On days 22 and 25 of an experiment, groups 1, 2 and 7 were injected by 30 mg/kg bw of diethylnitrosamine (DEN) via intraperitoneum for detecting anticlastogenicity. While groups 3 to 6 were injected by saline solution for studying clastogenicity. All rats were partially hepatectomized to amplify mutated hepatocytes at day 29. After operation for 4 days, the regenerated hepatocytes were isolated and the micronucleated hepatocytes were determined. The results showed rice husk extract did not induce number of micronucleated hepatocytes and mitotic index compared to a negative control (group 6). However, the extract significantly inhibited DEN-induced micronucleus formation in rat liver. Our results indicated that anthocyanin-rich extract from husk of purple glutinous rice variety Kham Doisaket might prevent the initiation stage of hepatocarcinogenesis in rats.

ARSENIC ACTIVATES MAPK SIGNALING IN HORMONE-DEPENDENT BREAST CANCER CELLS

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Arsenic (As) is a natural element which is considered as one of the top environmental health threats worldwide. Epidemiological evidence has associated exposure to arsenic in drinking water with an increased incidence of human cancers in the skin, bladder, liver, kidney and lung. Recently, several studies reported that arsenic is a potential environmental estrogen as it interfered with the action of estrogen (E2) and estrogen receptor (ER) signaling. In addition to classical genomic ER signaling, membrane ER (mER) and GPR30/EGFR/MAPK signaling has been recently found to mediate the rapid non-genomic estrogen signaling and was reported to participate in growth-stimulation of breast cells. Therefore, our study aimed to investigate whether arsenic activates proliferation of hormone-dependent (ERα positive) breast cancer cells by non-genomic ER/MAPK signaling pathway. Arsenic dose-dependently increased viability of hormone-dependent breast cancer MCF-7 and T47D cells but not hormone-independent breast cancer MDA-MB231 cells. Utilizing T47D-KBluc cells which derived from T47D cell stably transfected with estrogen responsive element (ERE)-luciferase reporter plasmid; effect of arsenic on ER transcriptional activity was studies. Arsenic treatment did not alter endogenous ER transcription activity whereas it dose-dependently reduced 17β-estradiol-induced ERE-luciferase activity. Previous reports have shown that exposure to E2 induced down-regulation of ERα protein expression. Therefore, modulation of ER expression was studied by Western immunoblot. Arsenic decreased ERα but not ERβ protein expression in both MCF-7 and T47D cells suggesting similar function of arsenic and steroid hormone estrogen on ERα. Further studies demonstrated that arsenic triggered rapid and sustained activation of MAPK (ERK1/2) which can be inhibited by MAPK inhibitor, U0126. These results indicate that in addition to affect on the genomic ER pathway, arsenic also activates rapid non-genomic signal transduction through ERK1/2 pathway which may contribute to its proliferative effect on hormone-dependent breast cancer cells.

ALCOHOLIC EXTRACT OF FERMENTED *HOUTTUYNIA CORDATA* THUNB INDUCES HUMAN LEUKEMIC HL-60 AND MOLT-4 CELL APOPTOSIS MORE STRONGLY THAN THE UNFERMENTED

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Houttuynia cordata Thunb (HCT) is a medicinal plant of Saururus family. It contains antimutagenic, antioxidant, anti-allergy, antiviral and antibacterial activities. In the process of preparing the HCT extract, the stems/roots/leaves were non-fermented or fermented with yeast, ethanol, then the supernate was removed and the stems/roots/leaves were dried, ground and extracted again with 95% ethanol or water. The aims of this study was to study the cytotoxic effect, type of cell death, and the mechanism of death compared between the alcoholic fermented/non-fermented and water fermented/non-fermented extract of the plants. The cytotoxicity was performed using MTT assay in human leukemic HL-60, Molt-4 and peripheral blood mononuclear cells (PBMCs). The apoptotic death was characterized by staining with propidium iodide and examined under fluorescence microscope. The peroxide radical production was determined by using 2',7'-dichlorofluorescein diacetate and flow cytometry. The reduction of mitochondrial transmembrane potential (MTP) was measured by using 3,3'-dihexyloxacarbocyanine iodide and flow cytometry. The caspase-9 and -8 expression was identified by immunoblot. It has been found that the ethanolic extract of fermented HCT was cytotoxic to HL-60 > Molt-4 > PBMCs. Whereas the ethanolic extract of non-fermented and the water extract of both fermented and non-fermented did not inhibit cell growth in HL-60, Molt-4 and PBMCs. The apoptotic HL-60 and Molt-4 cells increased dose dependently. The number of death cells treated with fermented HCT was more than that treated with non-fermented extract and the alcoholic extract treated apoptotic cells were more than the water extract-treated apoptotic cells. Peroxide radicals were produced also in a dose dependent manner. The alcoholic fermented extract produced more radicals than the non-fermented in HL-60 cells whereas the alcoholic non-fermented extract produced more radicals than fermented treated condition in Molt-4 cells. The reduction of MTP was found in HL-60 and Molt-4 cells treated with the alcoholic extract of fermented HCT. The proform of caspase-8 and -9 were more cleaved as the dose of alcoholic extract of fermented HCT increased in both HL-60 and Molt-4. In conclusion, fermented HCT extract was more toxic to the human leukemic HL-60 and Molt-4 cells more than the non-fermented and the cells underwent apoptosis mediated via the mitochondrial pathway.

Keywords: *Houttuynia cordata* Thunb, Fermentation, Mitochondrial pathway, Caspase-9, Caspase -8

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ESTABLISHMENT OF A DUAL ORGAN CARCINOGENICITY MODEL FOR EVALUATING CHEMOPREVENTIVE AGENTS IN RATS

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The general carcinogenicity tests are taking a lot of time and expensive because a larger number of animals are required. The anticarcinogenicity of natural products and synthetic compounds has long been evaluated. Most experimental protocols were modified from the single organ-specific carcinogenicity test. To reduce the rat number and cost of experiment, the dual organ carcinogenicity test was established. In this study, we have developed the liver and colon cancer in the same rat exposed to 2 carcinogens, diethylnitrosamine (DEN) and 1, 2-dimethylhydrazine (DMH), which have a similar metabolic pathway. The preneoplastic lesion in the liver, glutathione-*S*-transferase placental form (GST-P) positive foci and the preneoplastic lesion in the colon, aberrant crypt foci (ACF), were used as the endpoint markers of the early stages of liver and colon carcinogenesis, respectively. The rats were injected with carcinogens in different day, including injected with DEN before DMH, injected with DEN after DMH, and injected with DEN combination with DMH in the first injection. Eight weeks after the first injection of carcinogen, all rats were sacrificed and the number of GST-P positive foci and ACF were counted and compared to the control groups. The results showed that rats injected with DEN alone did not develop ACF while those injected with DMH alone did not develop GST-P positive foci. Moreover, the number of GST-P positive foci and ACF in rats injected with DEN and DMH were not differed from the control group. Interestingly, rats injected with DEN combination with DMH was significantly increased the GST-P positive foci when compared to DEN alone, but the number of ACF did not different with DMH alone. In conclusion, this dual organ carcinogenicity model might be an effective model for screening chemopreventive agents protected early stages of both colon and liver carcinogenesis in the same rat.

Keywords: Diethylnitrosamine, 1, 2-Dimethylhydrazine, Dual organ carcinogenicity test, Aberrant crypt foci, Glutathione-*S*-transferase placental form positive foci

HEAVY METALS, TOXIC AND ANTIOXIDANT TRACE ELEMENTS IN TOBACCO SMOKING

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Smoking is not only associated with decreased concentrations of several antioxidant vitamins and trace elements but also increased morbidity and mortality risk of diseases. Those due to heavy metal, other toxic and antioxidant trace elements in tobacco smoke are not sufficiently emphasized. Tobacco smoking influences on the concentrations of several elements in some organs. We sought to determine the relationship between the known effects of some trace elements and other biochemically important elements (Cd, Cr, Cu, Hg, Pb, Se and Zn) which are linked with smoking. Cigarette smoking may be a substantial source of intake of these hazardous elements not only to the smokers but also, through passive smoking, to nonsmokers. Studies were carried out on 150 smokers (50 industrial cigarette smokers, 50 passive smokers and 50 local tobacco smokers) compared with 50 nonsmoking controls. Levels of plasma lead (Pb) and cadmium (Cd) were significantly higher in smokers than in controls. Whereas chromium (Cr), selenium (Se), zinc (Zn) and mercury (Hg) levels were significantly lower among smokers than controls. For dietary intake assessment, smokers consumed significantly less energy from carbohydrate, fat compared to controls, while energy derived from protein did not differ between groups. Moreover, smokers consumed less dietary fiber and vitamins compared with controls. Increasing plasma toxic trace elements in healthy smokers may be explained by low antioxidant trace elements and vitamins that lead to develop oxidative stress and diseases and increased turnover or breakdown of vitamins and micronutrients. Therefore public health should not only aim for smoking cessation, but also concern about diet in terms of vitamin and trace element content.

Keywords: Smoking, Heavy metal, Toxic, Antioxidant trace elements

IN VITRO TOXIC EFFECT OF NANOSILVER ON PROGRESSIVE MOTILITY OF RAM SPERMATOZOA

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To determine the concentration/time dependent toxic effect of nanosilver colloid on progressive motility of ejaculated ram spermatozoa *in vitro*, sperm samples were incubated for 30, 60, 120 and 180 min in the presence of nanosilver colloid (0, 0.01, 0.1, 1 and 10 ppm). Sperm progressive motility was evaluated by computer assisted sperm analysis. It was observed reductions of sperm progressive motility in the most of treated groups compared to their corresponding controls which was significant ($P < 0.05$) especially in the concentrations of 1 and 10 ppm. Of course, at concentration of 10 ppm, spermatozoa were completely inactivated. This toxic effect of nanosilver was appeared after 30 min of experiment and has been observed in all times. It is concluded that nanosilver colloid discourages sperm functions specially its motility which can be a causative agent for sperm infertility.

LIMONENE ATTENUATES DOXORUBICIN-INDUCED RENAL TOXICITY VIA AMELIORATION OF OXIDATIVE STRESS AND INFLAMMATION

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Limonene is a naturally occurring flavone and has been found to posses numerous therapeutic properties. In this study, we used limonene as a protective agent against the nephrotoxic effects of anticancer drug Doxorubicin (Dox). Rats were given limonene at doses of 5 % and 10% mixed with diet for 20 consecutive days. Dox was give at the dose of 200 mg/kg body weight intraperitoneally. The protective effects of limonene on Dox-induced oxidative stress and inflammation were investigated by assaying oxidative stress biomarkers, lipid peroxidation, serum toxicity markers, proinflammatory cytokines, expression of NFKB, i-NOS and COX-2. Administration of Dox (200 mg/kg bwt) in rats enhanced renal lipid peroxidation; depleted glutathione content and anti-oxidant enzymes; enhanced expression of NFKB, i-NOS and COX-2. Treatment with limonene prevented oxidative stress by restoring the levels of antioxidant enzymes, further a significant dose-dependent decrease in inflammatory response and the kidney toxicity markers KIM-1, BUN, Creatinine and LDH was observed. Limonene also effectively decreased the Dox induced overexpression of NFKB, i-NOS and COX-2. Data from the present study indicate the protective role of limonene against Dox induced renal damage.

Keywords: Chemoprevention, Doxorubicin, Limonene, Nephrotoxicity

THE TEMPORAL CHARACTERISTICS OF SOD ACTIVITY AND MDA CONCENTRATION AFTER EXPOSURE TO PCB153 ON CULTURED RAT SERTOLI CELLS

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Polychlorinated biphenyls (PCBs) are considered as environmental contaminants of modern world, causing health hazards in human beings. Sertoli cells (SC) in the testis becomes one of the most important target cell of PCBs for its unique physiological function. The present studies were aimed at exploring the temporal characteristics of superoxide dismutase (SOD) activity and malondialdehyde (MDA) concentration after exposure to 2,4,5,2',4',5'-hexachlorobiphenyl (PCB153) on cultured rat SC. SC were isolated from 18- to 20-day-old male Sprague-Dawley rats. SOD activity and MDA concentration were determined after 6, 12, 24, 36 or 48 h treatment with 0 (solvent control, 0.3% DMSO), 10, 20, 30 μmol/L PCB153. After 24h exposure on cultured rat SC, PCB153 at 20, 30 μmol/L significantly decreased SOD activity, and increased MDA concentration as compared with the solvent control (P<0.05), with a dose-response relationship. As compared with the previous time point of the same dose, SOD activity were significantly increased after 12h exposure to 30 μmol/L PCB153 and 24h exposure to 20 μmol/L PCB153 (P<0.05), and were significantly decreased after 36h exposure to 10, 20 μmol/L PCB153 (P<0.05). Meanwhile, MDA concentration of cultured rat SC were significantly increased after 24h exposure to 30 μmol/L PCB153 and 36h exposure to 20 μmol/L PCB153 (P<0.05). Both of them had a time-response relationship at time points of 12, 24, 36 h. In short, SOD activity increased initially, and decreased subsequently, while MDA concentration increased continuously with time. At the same time point, SOD activity decreased and MDA concentration increased with the dose increase. Both of them had a dose- and time-dependent manner at 24h. These findings suggest that 24h was the best time point to determine oxidative stress indicators after exposure to PCB153 on cultured rat SC.

Keywords: 2,4,5,2',4',5'-hexachlorobiphenyl, Sertoli cells, Superoxide, dismutase, Malondialdehyde

IN VITRO CYTOTOXICITY OF MANGANESE DIOXIDE (MnO_2) DUST PARTICLES COLLECTED FROM A FERROMANGANESE SMELTER

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MnO_2 is described as an incidental nanoparticle which is created as an unintentional by-product in ferroalloy smelter works. Neurotoxicity of manganese has been shown to exhibit symptoms similar to Parkinson's disease; this form of the disease is called manganism. In the studies performed by J.E Myers group in 2003, on 200 randomly chosen production workers, no significant relationship was found between neurotoxicity endpoints and measurements of exposure, however there have been studies done conflicting these results. Since access of these nanoparticles to the brain is a possibility, in the present study, we would like to investigate the MnO_2 dust sample collected from a ferromanganese smelter in order to confirm its effects on neuronal cells. The physical properties of the MnO_2 dust were characterized i.e. size distribution, trace element analysis and surface area measurements. Cellular responses of primary rat astrocytes upon exposure to MnO_2 dust were studied by xCELLigence real-time monitoring technology and several biological endpoints were monitored i.e. cytotoxicity, uptake and levels of Nf-kappa B. For all experiments silica was used as the benchmark against which toxicity was measured. The MnO_2 dust was taken up by the astrocytes. The conventional cytotoxicity assays could not be used because of interference by the dust particles. Subsequently, the toxicity was determined using the xCELLigence system showing a concentration- and dose-dependent deviation in normal growth patterns. Changes in Nf-kappa B and cellular uptake assessed by the CytoViva Hyperspectral Imaging system were also observed. These results indicate direct disturbances in cellular toxicity, viability and markers of oxidative stress and inflammation.

OCCURRENCE OF TOXIC CYANOBACTERIA AND THEIR TOXINS FROM FRESHWATER BODIES IN VIETNAM – A SHORT REVIEW

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Blooms of cyanobacteria in freshwater bodies are a recurrent phenomenon throughout the world. Toxic cyanobacteria and their toxins (e.g. microcystins) are public health and environmental concerns because cyanobacterial toxins could cause acute and chronic poisonings to aquatic organisms and human being. Our paper reviews the occurrence of toxic cyanobacteria and their toxins from freshwater bodies, especially from drinking water supplies, in Vietnam. Toxic *Microcystis* is sometimes found with mass and blooms/scums growth in lakes and reservoirs in Vietnam. Approximately 1766 $\mu g.g^{-1}$dry weight (DW) and 4120 $\mu g.g^{-1}$ DW of microcystins was measured in cyanobacterial cultures isolated from freshwater bodies in Dong Nai Province and Hue City. In addition, the concentration of 2,857 $\mu g.L^{-1}$ microcystins was analyzed in surface water of Dau Tieng reservoir as well. These extremely high concentrations which go beyond the WHO guideline pose serious risks and hazards to millions of local residents who daily use water from the drinking water supplies consisting toxic cyanobacteria and microcystins for their domestic activities.

EFFECT OF LOW LEAD EXPOSURE DURING FOETAL AND EARLY POSTNATAL LIFE ON PRE-WEANING BEHAVIORAL TESTS AND POST-WEANING BEHAVIORAL TEST WITH A FOLLOW UP IN RATS

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Prospective studies in humans have confirmed cognitive and behavioral deficits in children with lead exposure, however information on long-term consequences in adulthood due to perinatal lead exposure are inconsistent. Objective of this study were (a) To study the spatial learning in rats at weaning and late adulthood after low-level lead exposure during their prenatal and early postnatal period,(b) To conduct pre-weaning behavioral test in developmental low-level lead exposed groups of rats. Male Wistar-derived rats (n=6) were exposed to lead (as 0.2 % lead acetate solution) through their mothers during pregnancy and lactation until they were weaned. Mothers of male control rats (n=6) were given tap water during pregnancy and lactation. All pups were weaned on tap water at 21 days of age and were followed up until 120 days old. Both groups of pups were subjected to preweaning behavioral tests (surface righting reflex test, negative geotaxis test and swimming development test). Morris water maze test (post-weaning behavioral test) was conducted on postnatal days 30 and 120. There was hastening in the day of negative geotaxis reflex development and impairment in swimming development in lead treated group of rats as compared to normal control rats. The Morris water maze test conducted on both postnatal day 30 and 120 showed impaired spatial memory in the lead exposed group of rats. To conclude, our study has shown that low-level lead exposure during the critical period of brain development and maturation can impair learning and memory in rats, long after the lead exposure had stopped.

OXIDATIVE AND INFLAMMATORY RESPONSES TO DIESEL EXHAUST PARTICLES (DEP) IN A549 AND RPMI1788 CELL LINES

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Particulate air pollution has become a major public health concern because epidemiological studies have demonstrated that exposure to particulate matter is associated with health effects such as respiratory diseases and cancers. Diesel exhaust particles (DEP) are considered a major contributor of the particulate air pollution, particularly in urban environments. The objective of the present study was to investigate biological effects induced by DEP on oxidative stress and inflammation in human alveolar epithelial cell line (A549) to compare the extent of responses with lymphoblast (RPMI1788). The oxidative responses were determined by measurement of reactive oxygen species (ROS) generation in different cell cycle phases and formation of DNA damages, including 8-hydroxy-deoxyguanosine (8-OHdG) and DNA strand breaks. The inflammatory responses were assessed by the change in gene expression of pro-inflammatory cytokines (*interleukin-6 and -8*; *IL-6* and *IL-8*) and anti-inflammatory proteins (*Clara cell protein*, *CC16*; *lung surfactant protein-A*, *SP-A*). The results show that DEP was internalized and induced ROS generation preferentially in G2/M phase as well as increased 8-OHdG in both A549 and RPMI1788 cells with a similar extent in a dose-dependent manner. In contrast, DNA strand breaks analyzed by the comet assay were higher in A549 cells. Moreover, DEP up-regulated the gene expression of pro-inflammatory cytokines in both cell types but down-regulated the gene expression of the anti-inflammatory protein (*CC16*) in A549 cells. In conclusion, A549 and RPMI1788 cells had a similar pattern of dose-dependent responses in terms of DEP uptake, ROS generation, formation of 8-OHdG and DNA strand breaks, and induction of the pro-inflammatory cytokine gene expression. This study suggests that circulating lymphocytes could be used as a surrogate sample for alveolar epithelial cells to assess possible health effects from DEP exposure in human biomonitoring.

MELATONIN ADMINISTRATION AMELIORATE TESTICULAR MITOCHONDRIAL OXIDATIVE DAMAGE CAUSED BY BISPHENOL A IN ADULT MICE

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Bisphenol A (BPA) is a monomer of polycarbonate plastic used to manufacture plastic baby bottles and lining of food cans. BPA is also used in dental fillings and sealants. It has endocrine-disrupting potential and exerts both toxic and estrogenic effects on mammalian cells. The aim of this study was to investigate if BPA induced oxidative stress and toxicity in the testicular mitochondria of adult male mice, is ameliorated by co-administration of melatonin. Mice exposed to standardized dose of BPA (10 mg/kg body weight), orally for 14 days caused lipid peroxidation (LPO) and decrease in reduced glutathione (GSH) content of testicular mitochondria. BPA also caused decrease in activities of marker mitochondrial enzymes such as succinate dehydrogenase, malate dehydrogenase, isocitrate dehydrogenase. Besides, it also affected activities of antioxidant enzymes such as superoxide dismutase, glutathione reductase and glutathione peroxidase. Concomitant melatonin administration (10 mg/kg body weight; intraperitoneally for 14 days) lowered mitochondrial lipid peroxidation. It also restored the activity of mitochondrial marker enzymes and ameliorated decreased enzymatic and non-enzymatic antioxidants of mitochondria. Melatonin acts as an antioxidant. These results demonstrate the prowess of melatonin in ameliorating BPA-induced mitochondrial toxicity and the protection is due to its antioxidant property or by the direct free radical scavenging activity.

ERADICATION OF TOXIC MATERIAL BY ASSESSED GREEN ORGANIC CHEMISTRY AS ANTICANCER AGENTS

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Cancer, a disease of the cell cycle, being one of the major health problems has received enormous biomedical attention over the past two decades. The effectiveness of many existing anti cancer drugs is limited by their toxicity to normal rapidly growing cells. Organic synthesis by non-conventional/modern methods is rapidly gaining importance in view of the fact that the use of many toxic and volatile organic solvents contributes to pollution. Consequently, it is highly desirable to develop environmentally benign processes that can be conducted in aqueous media/solvent-free/solid-supported and to minimize global warming and improve good health.In view of these points, it was thought worthwhile to study new benzimidazoles clubbed with fused heterocyclic ring systems; triazolo-thiadiazoles and triazolo-thiadiazines moieties, as rationally designed with bendamustine, solvent free under scientific microwave synthesizer and *In vitro* anticancer screening at the Development Therapeutic Program (DTP), National Cancer Institute (NCI), Chemotherapeutic Research division, USA, against full NCI 60 cell line panel. Compound 5h (NCS: 760452) namely 1-(1*H*-benzo[*d*] imidazol-2-yl)-3-(6-(2,4-dichlorophenyl)-[1,2,4]triazolo[3,4-b] [1, 3,4]thiadiazol-3-yl)propan-1-one, exhibited remarkable anticancer activity with Mean GI_{50} =1.04µM, TGI > 100 and LC_{50}> 100 in compare to standard drug (Bendamustine, NSC: 138783, Mean $GI_{50,}$ 60 µM, TGI>100 and LC_{50}> 100). Based on these observations, it could be a subject of further investigations for searching potential anticancer agents. Finally its conceivable that further derivatization of such compounds will be of interest with the hope to get more selective and potential anticancer agents.

PRELIMINARY STUDY OF PERSISTENT EFFECT OF LONG LEAD EXPOSURE ON ADULT RAT SKELETON

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Bone is recognized as the best storage tissue of lead; its half-life in bone is considered by years because it is bound tightly and is not available to other tissues (exception in some physiopathological states). Lead can substitute for calcium in the bones and causes metabolic problems when it replaces this element or another preferred mineral.The aim of this study is to investigate the persistent effect of long lead exposure on skeleton of adult's male and female Wistar rats. Two main groups of adult Wistar rats are used; intoxicated rats (n_{male}= 12, n_{female}= 12) received 50mg/L of lead nitrate diluted in tap water, while control rats (n_{male} = 12, n_{female}= 12) receive tap water only, for 6 months. The stopping of intoxication was for 4 months. At the end of this period, radiological examination of the skeleton of rats is realized. The preliminary results show that chronic lead exposure affects the rats' skeleton; the bone of ischium zone, in intoxicated rats, is too thin compared to the bone of control rats, in both sexes. However, the thickness is more important in female rats. Lead long exposure induces a diminution of bone density. However, deeper investigations (as bone densitometry or some blood analyses) are needed to well exploring this finding.

Keywords: Lead, Chronic exposure, Skeleton, Rat

EFFECTS OF FENOFIBRATE ON THE HEMODYNAMICS OF GLYCEROL-INDUCED RENAL FAILURE IN RATS

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The modulating effect of PPAR-α ligand on haemodynamic effects of PE, AII, ET1, Ach, SNP and Isoproterenol were evaluated in glycerol-induced acute kidney injury in rats. The effect of PE on fenofibrate treated animals was dose-dependent increase in MAP. For AII and ET1, though MAP was also increased for the fenofibrate group, but not in dose-dependent fashion. On the MBF, while the lower doses of PE and AII increased the perfusion unit on the fenofibrate treated group, the higher doses decreased the perfusion unit. The ET1 increased the perfusion unit on this group but not in dose-dependent fashion. The effects of PE and AII on the CBF of fenofibrate-treated group are similar to that of MBF for the same group but not for ET1. The effect of ACH, SNP and Isoproterenol for all the groups was that of decrease in MAP. Isoproterenol caused dose-dependent increase in MBF of fenofibrate-treated group. The effects of ACH, SNP and Isoproterenol on the CBF perfusion unit was that of increase for the fenofibrate treated group. The study showed that fenofibrate did not attenuate increased blood pressure induced by PE, AII and ET1 but caused enhanced vasodilation by Ach, SNP and Isoproterenol.

ASSESSMENT OF TITANIUM DIOXIDE CYTOTOXICITY ON NEURONAL CELLS IN CELL CULTURE

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Because of the broad range of possible nanotechnology applications, continued evaluation of the potential health risks associated with exposure to nanomaterials is essential to ensure their safety. Nanoparticles often exhibit unique physical and chemical properties that impart specific characteristics essential in making engineered materials, but little is known about the effects of titanium dioxide (TiO2) on the neuronal cells. The possible neurotoxicity of TiO2 on SHSY5Y cells (human neuroblastoma cell culture without microglia) was investigated. SHSY5Y cells were exposed to different concentrations of 10 and 25 nm diameter TiO2 NPs in different time periods. To determine toxicity levels of nanoparticles, cell viability was estimated by MTT test. Concentration of cells was assessed by counting trypan blue stained cells with a heamo-cytometer. Our study demonstrated that exposure of SHYS5Y to TiO2 NPs for 24 hours regularly induced reduction of cell viability. Thus TiO2 may be suspected for various neurological disorders. Further in vivo studies are needed to evaluate this assumption.

EVALUATION OF GENOTOXICITY IN INFERTILE MEN

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Today, the relationship between male infertility and genotoxic damage is an important topic to be studied on. It is well known that chemicals causes genotoxic damage in human genome of which sperm chromatin is also a part of that. The genotoxic damage on sperm causes defective sperm function, which leads to male infertility. Among all the molecular fertility biomarkers, the evaluation of sperm DNA damage is one of the most promising. Therefore, it is important to assess the level of genotoxic damage of the sperm chromatin with reliable biomarkers. Not only the DNA damage on germ cells, but also the accumulated DNA damage in somatic cells is also thought to effect on infertility. By applying the conventional biomarkers of genetic damage on somatic cells, early effects may be highlighted; thus genetic biomonitoring allows detecting adverse effects of mutagens. However, such an approach has not been widely used for developing strategies in risk assessment and disease prevention and no direct information can be drawn on germ cells. Therefore, it seems to be important to extrapolate the findings gathered in somatic cells to germ cells. For this purpose, we have evaluated the sperm chromatin integrity using one of the most commonly used cytogenetic biomarker, Comet Assay, on sperm samples of 82 infertile men and 63 healthy controls. As well as assessing the DNA integrity on sperm cells, we have evaluated the genetic damage on somatic cells, lymphocytes, from the same subjects to compare the possible relation of somatic-genetic interaction. All those results are compared with morphological and functional sperm parameters, which can be thought as the phenotype of sperm dysfunction and gives us the opportunity to search for the genotype-phenotype relationship of male infertility.

EFFECT OF ALCOHOL, CIGARETTE SMOKING ON SERUM GAMMA-GLUTAMYLTRANSFERASE LEVEL AND LIPID PROFILE IN THAI MEN

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Since the liver is the main organ that is responsible for metabolizing drugs, alcohol and other toxins, and it is particularly susceptible to damage associated to alcohol. Like alcohol, smoking has negative effects on the liver and can also provoke oxidative stress which is linked with lipid peroxidation. In addition, previous evidences suggested that smoking influenced on plasma lipoprotein concentrations and may thus mediate an important role in the development of coronary artery disease (CAD) risk. The objective of this study was to investigate the influence of alcohol consumption, cigarette smoking on alterations of serum gamma-glutamyl transferase (GGT) level and lipid profile in Thai population. Subjects included 278 Thai men, 30 to 60 years of age. Non-drinkers were 138 Thai men and drinkers were 140. Blood samples were collected and serum levels of GGT, total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), triglycerides (TG) were determined. The result showed the statistical difference in mean of GGT (39.93±2.69 vs 82.93±7.28 U/L, $p<0.001$), HDL (50.52±0.98 vs 55.76±1.32 mg/dL, $p<0.05$) and LDL (143.50±3.10 vs 128.77±3.02 mg/dL, $p<0.05$) levels between non-drinkers and drinkers. Further analysis with cigarette smoking, the significant changes in these parameters also found both in dependent and independent manner. The results from present study suggested the important behaviors such as alcohol intake and cigarette smoking on the potential risks of liver and cardiovascular diseases.

NANOMATERIAL TOXICOLOGY - IMPORTANCE OF CHRONIC TOXICITY ASSESSMENT

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Asbestos (mesothelioma/ lung adenocarcinoma) and Thorotrast (3-10 nm-sized thorium dioxide; reticuloendothelial system (RES) deposition with in vivo half life of over 20 years) are the two of a few human examples of chronic Particulate Matter (PM) Toxicity. Some multi-wall carbon nanotubes are 10 to 20 micrometers long and induce mesothelioma when administered intraperitoneally (i.p.) to p53 heterozygous mice. A low dose i.p. study suggested the non-granulomatous chronic inflammatory micro lesions as the primary foci for a typical mesothelial proliferation. Incidental findings in this study were systemic distribution of single MWCNT fibers probably via blood stream. Some of them are found to be trapped by the RES.Fullerene molecules form micrometer-sized aggregates by van der Waals force. An i.p. study of fullerene indicated that the phagocytic cells seem to "digested" the aggregates down to sub-micrometer granules and bring them to RES. Currently, these two cases of PM toxicity response are the initial keys to consider chronic toxicity of a new nanomaterial. Information of pulmonary fibrosis and/or systemic distribution without overt acute and chronic inflammatory reactions is accumulating. In general, acute response is not a predictor of chronic toxicity of biopersistent nanoparticles. Pathology of chronic effects, possibly submicroscopic, should be studied in a case-by-case basis for a while. To maintain a sound activity of industrialization of nanomaterials, timely transfer of information on the chronic toxicity of the new materials or the products to the manufacturers would be of great importance, especially before the product development and mass production. In order to facilitate such process, development of appropriate toxicology scientists who are able to predict such “new” chronic toxicity by making full use of traditional as well as recent methodologies should be promoted and supported not only by the government but also by the private sector.

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OCTYLPHENOL AND TRICLOSAN HAVE STRONG STIMULATORY EFFECTS ON THE PROLIFERATION OF HUMAN BREAST CANCER CELLS VIA AN ESTROGEN RECEPTOR-MEDIATED SIGNALING PATHWAY

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The class of endocrine disrupting chemicals (EDCs) is comprised of both naturally occurring and man-made compounds, which can interfere with the actions of an endogenous steroid. Octylphenol (OP) is alkyl phenol chemical, which is considered to be a low-level endocrine disruptor owing to its tendency to mimic estrogen. In addition, recent studies have reported that triclosan has potency to endocrine disruption and has weak estrogenic activity with binding affinity to ERs. In this study, we examined that these EDCs, which have an estrogenic activity, can induce cell growth of human breast cancer cells via an ER-dependent signaling pathway by using the cell proliferation assay, semi-quantitative RT-PCR and Western blot analysis. Treatments of MCF-7 breast cancer cells with OP and triclosan strongly resulted in the stimulation of their cell gorwth and induced the alteration of transcriptional and translational levels of cell cycle-related genes, *cyclin D* and *p21*. These OP and triclosan increased the proliferation of MCF-7 breast cancer cells in a dose-dependent manner, similar to 17-beta estradiol (E2). We also found that they caused the induction of *cyclin D1* gene and reduction of *p21* gene at tranlational levels. It can be assummed that the alterations of genes, during the G1/S transition, may lead to increase cell proliferation in MCF-7 cells by OP and triclosan. However, cell growth and alteration of genes caused by OP and tricalosan can be reversed by reduction of ER signaling. To ensure the effect of these EDCs on ER□, we knowdowned ER□by siRNA in MCF-7 cells and treated the both ICI 182,780, in these cells. An activation of an ER□ signaling by propyl pyrazoletriol (PPT) can promote the effect of OP and triclosan on the dysregulation in cell cycle. Taken together, OP and triclosan can lead to stimulate breast cancer cell growth via the alterations of *cyclin D1* and *p21* genes in human breast cancer cells through an ER-mediated signaling pathway. A further study is required to determine the effects of EDCs on breast cancer carcinogenesis *in vivo*.

Keywords: Breast cancer, bisphenol A, methoxychlor, triclosan, estrogen receptor

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TREATMENT WITH BENZOPHENONE-1 RESULTED IN THE CELL GROWTH OF BG-1 OVARIAN CANCER CELLS WITH ESTROGEN RECEPTORS VIA REGULATION OF CELL CYCLE-ASSOCIATED GENES

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Benzophenones are organic chemicals widly used as stabilizers to UV light irradiation in plastic surface coatings and food packaging. They have been known that thier derivatives appear to have cytotoxic effects. 2-Hydroxy-4-methoxybenzophenone (Benzophenone-3, BP-3) and its metabolite, 2,4-dihydroxybenzophenone (Benzophenone-1, BP-1), are used mostly in the formulation of nail polishes, enamels, bath products, sunscreens and skin care products. In this study, the estrogenic effects of BP-1 were examined in an ovarian cancer BG-1 cells expressing high levels of estrogen receptors (ERs) by an cell viability assay, semi-quantitative reverse-transcription PCR (semi-quantitative RT-PCR) and Western blot analysis. The treatment of BG-1 cells with BP-1 (10^{-8}~10^{-5} M) resulted in an increase in their cell proliferation as 17-beta estradiol (E2) did. But, the cell growth stimulation by BP-1 was reversed by cotreatment with ICI 182,780, an ERs antagonist, suggesting that the proliferation of BG-1 cells is mediated by an ER-dependent pathway. In addition, BP-1 upregulated the expression levels of cell-cycle regulating genes, i.e., cyclin D1, which is a downstream target of ER, at 6 h after treatment. But, the expression of p21 gene was not altered by BP-1 at any time points. In translational levels, BP-1 also upregulated the expression level of cyclin D1 at 24 h after its treatment. But, upregulated expression of cyclin D1 by BP-1 was reversed by cotreatment with ICI 182,780, at both mRNA and protein levels. Taken together, these results suggest that BP-1 is one of EDCs with have apparent estrogenic activities by stimulating cell proliferation of BG-1 cells and by inducing the expression of cyclin D1. Our results can support that BP-1 may have a potency to disrupt endocrine system and to stimulate cell growth in ER-positive cancer cells.

Keywords: Ovarian cancer, benzophenone-1, estrogen receptor and estrogen

This work was supported by a National Research Foundation of Korea (NRF) grant funded by the Ministry of Education, Science and Technology (MEST) of Korea government (no. 2011-0015385).

PHYTOPLANKTON DISTRIBUTION AND QUANTIFICATION OF MICROCYSTINS IN SPECIFIC HYPERTROPHIC MAN-MADE DAMS IN THE KRUGER NATIONAL PARK, SOUTH AFRICA

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In the last decade, an increase in the formation of algal blooms has been observed in some of the man-made dams at the Kruger National Park (KNP). The main aim of this study was to investigate the limnological factors that influence the phytoplankton distribution and also to detect and quantify microcystin (MC) toxins present in the six KNP dams. The study was conducted between 2009 and 2011 (June 2009, September 2009, February 2010, July 2010, October 2010 and February 2011) and the parameters that were measured include: algal biomass, water temperature and pH, dissolved oxygen, total phosphorus (TP), total nitrogen (TN), turbidity, total dissolved solids and microcystin concentration (ELISA and HPLC). The phytoplankton were generally dominated by Cyanophyceae (*Microcystis*, *Anabaena* and *Oscillatoria*) and Chlorophyceae (*Chlamydomonas*, *Closterium* and *Crucigenia*). A multilinear regression analysis showed that the abundance of algae was positively correlated with the TN, TP and turbidity. In addition, MC toxins (-LR, -RR, -YR) were positively correlated with the algal biomass, TN and TP. In the KNP dams, TN and TP were the principal parameters driving the formation of algal blooms. Presence of the seven MC toxins (MC-RR, -YR, -LR, -LA, -LW and -LF) varied among the six dams. The most dominant variant was MC-LR which was present in all six dams. Overall, this study advances our understanding of the parameters playing a critical role in eutrophication at the KNP.

HEALTH RISK ASSESSMENT ON OCCUPATIONAL HAZARDS EXPOSURE AMONG HOME WORKERS INVOLVED IN THE TEXTILE INDUSTRY: A CASE STUDY OF FISHNET PRODUCTION, THAILAND

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Previous studies have reported that informal sector workers have potential health risks from work environmental hazards which could affect the quality of their life, family and society. Risks can arise in every occupation especially in home workers involved in textile manufacture. This case study aimed to determine the health risk of exposure to occupational hazards among home workers in Banthum municipality, Khon Kaen province, Thailand. Fishnet is the textile production in this community. Data were collected by in-depth interviews and observations at workplaces. Workers involved in the process of the fishnet production at home were classified into three groups which were material handling (n=42), lead attachment (n=42), and float attachment (n=42). The qualitative health risk assessment of exposure to occupational hazards was performed by considering the working procedures of each work type, and possible work hazards were identified. Risk was characterized from the outcomes of likelihood level and severity level of hazards. Workers involved in material handling had the highest risk of ergonomic hazard: awkward postures (52%), followed by materials that caused injuries (24%) and lead poisoning (22%). Workers of float attachment had the highest risk of ergonomic hazards: awkward posture and repetitive work (57%), followed by inappropriate tools causing injuries (21%) and chemical poisoning by skin contact (10%). Workers of float attachment had the highest risk of awkward posture and repetitive work (66%), followed by materials that caused injuries (29%) and insufficient lighting conditions (5%). Therefore, home workers should be aware of occupational hazards, and should use personal protection equipment for better protection. There should be a training and surveillance program on occupational health to home workers for safe working and living.

REMOVAL OF FORMALDEHYDE AND BENZENE BY COMMON TROPICAL PLANT SPECIES

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Indoor-potted plants have an important role in the removal of air borne contaminants including dust, inorganic gases and VOCs. In this study, VOCs removal rates of seven tropical plants were evaluated. The common tropical plants were selected namely, four species of floral plants, *Euphorbia milli Desmoul, Jasminum sambac, Ixora coccinea L, Anthurium spp* and three species of green leaf plants, *Golden pothos, Syngonium podophyllum,* and *Dieffenbachia,* Various doses of carcinogenic VOCs, formaldehyde and benzene were tested with and without plants in sealed chambers. Total VOCs concentrations were measured by Indoor air quality gas sensor, photo ionization detector (PID) with detection limit 10 ppb. All plant samples showed VOCs removal ability. For benzene, *Euphorbia milli Desmoul* was found highest removal rate 55.56%. The removal rate of formaldehyde was 84.83% in *Anthurium spp* and 60.51% in *Syngonium podophyllum*. With respect to the growth media, general plant soil showed some capacity with 9% benzene reduction and 5% formaldehyde reduction. This finding demonstrated the application of pot plants in the contribution of cleaner air environment which could be developed to plant-based biofiltration system in the future.

Keywords: Indoor air, Pot plant, VOCs, Benzene, Formaldehyde.

FORMER DENIM SANDBLASTERS WITH SILICOSIS REVEALED GENOTOXIC EFFECT IN TARGET AND SURROGATE TISSUES

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Occupational exposure to the crystalline silica (quartz) due to denim sandblasting is a crucial problem in Turkey. Although the procedure has been banned, reports on the silicosis development and other clinical outcomes among many former workers still remain. Our aim is, to investigate the genotoxicity in the former denim sandblasters with silicosis. As the first part of an ongoing project on former denim sandblasters; 15 silicosis patients diagnosed according to the X ray chest films have been engaged to the present study. The control group consisted of 15 healthy volunteers. Cytokinesis Blocked(CB) Micronucleus (MN) Assay in peripheral blood lymphocytes (PBL) and MN Assay in cells from sputum induction (SI) were carried out. The workers had 3.77±1.82 (mean ±sd) years sandblasting history and they have been given up for 5.73±1.49 years. According to the ILO classification; the numbers of silicosis patients were 9 in Group III, 3 in Group II and 3 in Group I. The CBMN frequency (‰) of workers (4.33±2.16) were higher than that of the control group (1.73±1.71) ($p<0.05$). In the same manner, SI-MN frequency (‰) of workers (11.00±4.74), were found to be increased against to the control group (3.78±2.39) ($p<0.05$). Furthermore, there was correlation between the IS-MN frequency and grade of silicosis ($r=0.705$, $p=0.002$). As a conclusion it is the first study using SI-MN assay regard to quartz exposure. It has been established that the genotoxic risk increased in fomer denim sandblaster workers with silicosis. These preliminary results may support the research on the likely interaction between the silicosis and lung cancer development.

DETERMINATION OF LEVELS OF PAHs, PCBs AND ORGANOCHLORINE PESTICIDES IN HUMAN BREAST MILK IN MERSIN CITY, TURKEY

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Analysis of environmentally related chemical contaminants in human breast milk provides a means not only to assess the contaminant burden in mothers but also to assess potential exposure of neonates feeding on breast milk. There are limited data on levels of PCBs and organochlorine pesticides (OCPs) in humans but there is no previous reports from Turkey on chemically determined levels PAHs in human breast milk. The main purpose of this study was to report the levels and accumulation profile of OCPs, indicator and dioxin like PCBs and 16 EPA priority PAHs in Turkish breast milk samples obtained from a Mediterranean city, Mersin,Turkey. We analyzed the presence of OCPs, PCBs and PAHs in breast milk collected from 47 individual samples from volunteers recruited from the census. Analysis were performed by use of a gas chromatograph coupled to a mass spectrometer (GC–MS). p.p'-DDE was the dominant pollutant. ß-HCH, p.p'-DDT, dieldrin, hexachlorobenzen, oxy-Chlordan, cis-Heptachlorepoxide were the other main organochlorine pesticides detected. Mean levels of ΣPCB congeners and $WHO_{PCB}TEQ$ were 9.94 and 0.001 ng/g lipid, respectively. PCB 153 showed the highest concentration (3.37 ng/g lipid), followed by PCB 138 and 180. For the dioxin-like PCBs, PCB 118 was the dominant (0.97 ng/g lipid). Naphthalene, phenanthrene, pyrene and fluoranthene were the major PAHs among the 16 PAHs detected. The estimated daily intakes of DDTs, PCBs, HCHs and HCB were not exceeded the tolerable daily intake (TDI) proposed by the Health Canada Guideline. Our results indicate that the neonates born in Mersin province are less exposed than the ones born in other regions, considering OCP and PCB levels in breast milk.

ASSESSMENT ON HEALTH RISK OF BENZENE EXPOSURE AMONG WORKERS AT GAS STATIONS IN MUANG KHON KAEN: A PILOT STUDY

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Workers at the gas station are potentially exposed to benzene from gasoline and automobile emission in a higher level than exposures among the general public. This pilot study aimed to investigate the health effect, the protection behavior and the potential risk of benzene exposure among workers at gas stations. Participants were 31 workers at gas stations in Muang Khon Kaen Municipality, Thailand. Data were collected by an applied survey form of health risk assessment on work hazards exposure. The potential risk level was determined following the objective and subjective adjustments. The resulted showed that most workers at gas stations were male (77.42%), age 15-24 years (58.06%) and work experience <1 year (51.61%). All workers reported mild symptoms of health effects from benzene exposure, which were vertigo (67.74%), fatigue (54.84%) and headache (51.61%). The moderate severity of reported symptoms were blur conscious (41.94%), depress/confuse (25.18%) and chest pain (22.58%). Severe symptoms of pale (16.13%) and convulsion (3.23%) were also reported. Unsafe behaviors of most workers were working without personal protective equipments (67.74%), eating or having meal in workplace (45.16%) and eating in working time (32.16%). The probability level of benzene exposure determined from hazard conditions and protection behaviors identified that 19.87% of workers were at high potential level and 83.87% were at medium level. The potential health risk on benzene exposure, considering the signs of health effect and the probability level of benzene exposure, identified that 9.68% of workers had high potential risk level and 41.93% were at rather high risk level. Therefore, workers at gas stations should be aware of benzene toxicity and better protection. The further investigation of health risk assessment on benzene exposure is suggested.

RISK DETERMINATION ON PESTICIDE EXPOSURE BY BIOLOGICAL MONITORING AMONG AGRICULTURISTS: A CASE STUDY IN TAMBON KANGSANAMNANG, NAKHONRATCHASIMA PROVINCE

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The cross-sectional analytic research aimed to determine the risk level of pesticide exposure by blood cholinesterase enzymes indicator and to study the risk factors for pesticide exposure among farmers in Tambon Kangsanamnang, Kangsanamnang District, Nakhonratchasima Province (n=110). The study was conducted in April 2011 by face-to-face interviews with the structural questionnaires included the knowledge, behavior, attitude and social support of using pesticide. All participants were monitoring for blood cholinesterase enzymes levels with the reactive paper. The results of blood cholinesterase identified that 27.3% of farmers were risk detected at unsafe level, 32.7% were at risk level, 30.9% were detected at safe level and 9.1% had normal blood cholinesterase enzymes. Factors associated with the risk levels of pesticide exposure were identified by inferential statistics using Chi-squared test. Data showed no statistically significant association of the studied factors and risk level from blood test. However, it was noticeable that factors of age, the period of pesticide use, the knowledge and the behavior of farmers had trends of relation to risk level of the blood cholinesterase. From this study, most agriculturists were at risk and unsafe level of pesticide exposures. There should be repeated test of red blood cell cholinesterase in the laboratory for the risk group. To prevent chronic exposure and the adverse health effect, health care center together collaborated with local administrative organization should provide the health surveillance program and pesticide exposure monitoring annually in agriculturists. A decrease of using pesticide and a better protection by using personal protective equipments are very necessary for agriculturists to reduce the risk of pesticide exposure. The results are useful for health risk assessment on a specific type of pesticide exposure among farmers.

ENVIRONMENTAL AND HEALTH RISK DERIVED FROM HANDLING OF EMPTY PESTICIDE CONTAINER

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At present, Thai farmers always sell empty pesticide containers to recycling business. On one point of view, this has made more economic benefit from waste, and retarded natural resources consuming by human activities. But on the other hand, this is one of the ways dispersing pesticide residues to the environment vastly. This study was carried out to confirm the way of prevention the distribution of pesticide residue into environment by means of rinsing empty container with water. Pesticide residue analyses in pesticide containers collected from farmers with general practicing indicated high level of pesticide residues, i.e. up to 20.85 and 415.07 mg/l for chlorpyrifos and glyphosate, respectively, which reflects higher risk of pesticide distribution in broaden areas and adverse impact to human health and the environment. Modified pesticide container rinsing method (3-rinse method suggested by WHO) showed a potential of practical and possible way to apply. Strongly shaking empty containers with 1/3 parts of clean water for 30 seconds at least 4-5 times has resulted in less than 1 mg/l of pesticide residues. Public information and education on properly rinsing method as well as adverse impact to human health by pesticide contamination in the environment should be taken into account by the governmental policy and enforcement into implementation in order to promote behavioral changing to the time consuming practice.

Keywords: Pesticide containers, Recycling business, 3-rinse method, Chlorpyrifos, Glyphosate

COPPER STATUS AND CADMIUM EXPOSURE OF PREGNANCY LIVING IN ENVIRONMENTAL CADMIUM POLLUTION

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Copper is an essential trace element that is vitally important for health. Deficiency of copper can have the following symptoms in human beings anemia, osteoporosis, elevated cholesterol levels. In cadmium contaminated area people have both beneficial and harmful elements in our daily lives. Copper and cadmium as well as divalent metal the transfer may competition of transporter such as divalent metal transporter 1(DMT 1). Cadmium is widespread, highly toxic metal pregnant women living in contaminated area are high risk. The aim of study to compared copper levels of the cadmium exposure among pregnant women living in cadmium contaminated area with pregnant women living in non-polluted area. Blood samples were collected at 36-38 weeks of gestation from pregnant women (non smokers) who delivered at Mae Sot General Hospital and lived in 12 villages of polluted area (cases, n=22) and non-polluted area (control, n=25). Blood cadmium and copper were determined by graphite furnace atomic absorption spectrophotometer (GFAAS). The results exhibited a significant high cadmium level in blood ($p=0.01$) of pregnancy women living in polluted area (1.3±1.0 µg/l) when compared to the control group (0.6±0.5 µg/l). The copper levels of cases group and control group were 1.44 ± 0.22 and 1.43 ± 0.44 mg/l, respectively ($p>0.05$). The results from this study suggested that cadmium exposure of pregnant women may be associated with living in cadmium polluted area while similar levels of copper were found in both groups and the status were within the normal range (0.7 - 1.6 mg/l). Copper status of pregnant women may not be involved to cadmium availability.

THE USE OF CADMIUM CONCENTRATION IN PLACENTA AS BIOMARKER OF CADMIUM EXPOSURE IN PREGNANT WOMAN: A STUDY OF THE ACCUMULATION IN DIFFERENT POSITION OF PLACENTAL TISSUE

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Cadmium is a toxic metal of considerable occupational and environmental concern. Exposure to cadmium has been linked to renal dysfunction, cancers, hypertension and various diseases. Environmental contamination of cadmium causes a food-chain transfer and bioaccumulation in most human foodstuffs and considered as a major route of exposure. Mae Sot District, Tak Province, the paddy fields receiving irrigation from the two creeks and crops grown in the areas were found to contain markedly elevated cadmium levels. The aim of this study was focused on the accumulation of cadmium in two different positions of placental tissue, beside umbilical cord and a margin of placenta and comparison between two groups from pregnancy women living in the contaminated area and non-contaminated area in Mae Sot District. The analysis of cadmium was determined by Graphite Furnace Atomic Absorption Spectrophotometry (GFAAS) and Inductivity Coupled Plasma Mass Spectrometry (ICP-MS). Our preliminary results showed the mean of cadmium concentrations in placental tissue were significantly greater ($p < 0.05$) in pregnancy women living in contaminated area than non-contaminated areas in both sample sections. The levels of cadmium in placenta tissue sample collected from beside of umbilical cord were higher than the margin area however, the concentrations detected in two different region showed non-significantly difference ($p > 0.05$). Since our previous results revealed that the levels of cadmium in three types of specimens, blood, urine and placenta tissue showed the significant correlations indicating that concentration of cadmium in placenta could be used as biomarker of cadmium exposure in pregnant women however, our present data suggested that the further analysis in more different locations of tissue sampling have to be conducted for giving final conclusion of recommended sampling area for the assessment of cadmium exposure in pregnant women.

TOXIC CYANOBACTERIA FROM TRI AN RESERVOIR, VIETNAM, AND THEIR ADVERSE IMPACTS ON DAPHNIA MAGNA

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Tri An Reservoir provides drinking water for millions of people in Southern Vietnam. Due to its mesotrophic status, cyanobacteria were monitored, isolated and cultivated for toxin analysis. Simultaneously, toxicity of cyanobacteria was tested with *Daphnia magna* at different aspects including enzymatic, behavioral responses and life traits of the animals. Daphnids were exposed to crude extracts of cyanobacterial isolates or cultures for enzyme activity or behavioral change studies. Animals were also exposed chronically for two generations to microcystin (MC) in 5 or 50 µg L^{-1} starting at neonate stadium. Life history traits were observed for the first generation for two months and the second generation for one week. Twenty seven species of cyanobacteria were recorded and *Microcystis* was common in the reservoir. Total MC concentration in scum samples was up to 0.64 mg g^{-1} dry weight. Cyanobacterial biomass temporally and spatially varied ranging from 0.009 – 0.834 mg L^{-1}. Isolates caused sudden changes in animal behavior and crude extracts induced an increase of antioxidant enzyme activities of daphnids. Survivorship decreased during chronic exposure with increasing MC concentration. Low concentrations of MC slightly affected the growth and reproduction of parent daphnids. However, high concentrations caused a strong reproduction reduction. Age to maturity of the offspring increased and their survival decreased after parent generation was exposed to the toxin, even if the offspring were raised in control medium. Growth of offspring declined with increasing toxin concentration. Moreover, cessation of the eggs/embryos was observed and malformation of neonates caused by cyanobacterial toxins was firstly recorded.

MONITORING RURAL WORKERS EXPOSURE TO PESTICIDES BRASILIA, BRAZIL

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The aim of this study is to present the results obtained while monitoring the exposure of rural workers, through analysis of butyrylcholinesterase levels, from 2009 to 2010, of 10 rural areas located in Brasilia, Brazil. Cholinesterases are enzymes that hydrolyze acetylcholine and other choline esters. They are divided in two classes: acethylcholinesterase, the real cholinesterase (erythrocyte), and the butyrylcholinesterase, pseudocholinesterase (plasma cholinesterase). In the presence of inhibitors, the butyrylcholinesterase is depressed and it is recovered before the erythrocyte. Organophosfates consist of a group of chemical compounds largely used in farming as pesticides. They act inhibiting cholinesterases, mainly acethylcholinesterase. The data analyzed was gender, age and blood level. It was observed that 93,3% were males, 63,1% were adults from 30 to 59 years old and 11,2% were below the preconized level. Pesticides are widely used in Brazil therefore occupational monitoring is a very efficient way to prevent and diagnose intoxications caused by these xenobiotics.

OXIDATIVE STRESS STATUS AND ACETYLCHOLINESTERASE ACTIVITY IN FARMERS EXPOSED TO ORGANOPHOSPHATE PESTICIDES IN NIGERIA

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Organophosphate agents constitute about half of all pesticides used globally and they appear to pose the greatest risk among all the pesticides. Despite significant advances in the understanding of the potential mechanism of toxicity far beyond the acceptable mechanism of cholinesterase inhibition in intentional exposures, the precise health effects following occupational exposures are yet to be completely defined. Oxidative stress status and Acetylcholinesterase activities were studied in blood samples obtained from 25 farmers in Idi Ayunre, Oluyole local government area of Oyo State, using organophosphate (OP) pesticides in spraying their cash crops (cocoa and cola nut trees) with a minimum work history of 10 years, in the age range of 35-75 years. 20 age-matched workers, who never had any exposure to OP pesticides were selected as controls in Ibadan, the capital of Oyo State. Total Plasma Peroxide (TPP) levels using FOX-2 reagent, Total Anti oxidant potential (TAP) using the ferric reducing antioxidant power (FRAP) assay were determined and oxidative stress index (OSI) an indicator of oxidative stress status was calculated. Blood acetylcholinesterase activity was measured using HPLC. Statistically significant decrease in the mean blood levels of acetylcholinesterase(IU/L) in the farmers(43.35-/+9.07) compared to the controls(65.28-/+7.66). TPP (umolH2O2/L) increased significantly in the farmers(14.32-/+5.18) than in the controls(10.25-/+3.60) ($p<0.05$), while depletion of TAP(umolTroloxequiv/L) was observed in the farmers(915.65-/+130.16) than the controls(975.80-/+142.70). OSI(%) in farmers(1.65-/+0.69) increased significantly than controls(1.08-/+0.39). OP pesticides users are exposed to increased oxidative stress. Assay of acetylcholinesterase activities could be a good biomonitoring index.

Keywords: Acetylcholinesterase, organophosphate pesticides, oxidative stress

DETERMINATION OF CADMIUM, MANGANESE AND LEAD LEVELS IN BLOOD AMONG ELECTRICAL ARC WELDERS

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Cadmium, manganese and lead in welding fume have been reported to have adverse health implications. The aim of this study is to assess the blood levels of cadmium, manganese and lead among electric arc welders. This study was conducted on 32 male workers in welding shops in Thasala and Nakhon Si Thummarat Municipality, Southern Thailand. They were adult men aged between 20-45 years (mean=27.5±5.8 yrs), working 8 hours/day with one day off per week. Mean employment duration ranged from 2-20 years (mean=5.5±2.8 yrs). The control group was 35 males who did not have any exposure to welding fume before. Both exposed and control groups were similar in age, sex, socio-economic status, smoking habits. The levels of cadmium, manganese and lead were determined in blood by atomic absorption spectrophotometry. Cadmium, manganese and lead concentrations in blood of welders were 5.5±1.8 µg/L, 15.2±5.7 µg/L, and 0.72±0.12 µg/L, respectively. Interestingly, blood cadmium and manganese levels in welders were significantly higher than in the control group *(P<0.01)*, although no statistical difference in lead level was observed in blood between the two groups. These results indicated that the high levels of blood cadmium and manganese in welders might be due to occupational exposure caused by improper use of personal protective equipment, lack of local exhaust ventilation system and poor personal hygiene habits. In addition, the concentration of cadmium in blood among welders exceeded the standard blood cadmium value cited from the ACGIH biological exposure indices (BEI) which could result in renal dysfunction in welders. This preliminary study showed current situation in cadmium exposure among welders in Thailand. Further studies should expand on this investigation by studying a larger number of welders, cumulative cadmium retention, bioelements profile, and environmental monitoring in different areas in the welding shops in order to prevent adverse health effects among the welders.

RELATION OF HYPERLIPIDEMIA AND MALONDIALDEHYDE LEVEL IN URBAN POPULATION

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Nowadays, increasing trend in burden of disease and illness caused by chronic non-communicable diseases and health risk behavior is an important health problem for urban population. Many types of chronic diseases were caused by lipid peroxidation process. The aim of the study to determine the relation of hyperlipidemia and lipid peroxidation biomarker, malondialdehyde (MDA) level, and various factor related to MDA levels in urban population. All participants (n=268) represent as the people who lived in three urban population such as Suan-gean community (Bangkok) (n=77), Wat Kai Tae community (Bangkok) (n=62) and Bang Boe community (Samutprakarn province) (n=129) were established. Fasting blood sample were taken and information about the family history; medical history; environment exposure; and food frequencies intake were collected. Plasma level of total cholesterol (TC), HDL-cholesterol (HDL-C), LDL-cholesterol (LDL-C) and Triglycerides (TG) were analyzed. MDA levels were determined by High Performance Liquid Chromatography (HPLC). The results showed hypercholesterolemia was found in 72% of total population. High LDL-C level (70%) and high TG (13%) were found. The mean plasma MDA level was 0.255 ± 0.092 μmol in healthy population. The MDA levels in female (0.318 ± 0.123 μmol) were higher than male (0.282 ± 0.098 μmol) in total population. There were no relation between hyperlipidemia condition and MDA levels. Hyperlipidemic patients, ranged in age from 35 years to 60 years have an inversely related with MDA levels ($p \leq 0.05$). Interestingly, correlation of lipid profile and MDA was found positive in patients who suffer from disease condition more than one type which have normal HDL-C levels but high LDL-C ($p < 0.05$). Another factor such as BMI, gender, smoking habit, alcohol consumption, and exercise were not related with MDA levels. We conclude that hyperlipidemia was not related with MDA level in urban population.

ISCHEMIC HEART DISEASE RISK FACTORS IN LEAD EXPOSED WORKERS

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Background: Some controversial epidemiological studies have shown that there are relationship between high blood lead level and risk of hypertension, hyperlipidemia and hyperglycemia. In this study we wanted to show if there is relationship between blood lead level and these ischemic heart disease risk factors. **Methods:**This study was conducted in a battery recycling plant and 497 male workers with the mean age of 41.7 (±6.50) years were recruited from all over the plant. Personal information such as demographics and work history was obtained through a questionnaire. Mean (±Standard deviation) For quantitative variables , Frequency (Percent) for qualitative variables , and Odd's ratio (OR) with 95% confidence interval (95% CI) for estimating the effect of blood lead level on lipid profile[triglyceride (TG), cholesterol(CHOL), low density lipoprotein –Cholestrol(LDL-C),high density lipoprotein –Cholestrol(HDL-C)], hypertension(HTN) and fasting blood sugar (FBS) level. Logistic regression modeling was used for multivariate analysis and adjusting the effect of different variables (age,body mass index(BMI), eating habits, cigarette smoking,etc**). Result:**The mean Blood Lead Level (BLL) was >40µg/dl in 281 (56.6%) subjects, ≤ 40µg in 216 (43.4%) subjects and the mean BLL was 43.3µg/dl (n=497). The mean job experience involving lead exposure was 13 years. There was no significant correlation between BLL and FBS (p=0.68), between BLL and TG (P=0.32), between BLL and HDL-C (p=0.49), between BLL and LDL-C (p=0.17), between BLL and CHOL(p=0.96), between BLL and systolic blood pressure (p=0.12).The adjusted Odd's ratio for the effect of BLL >40.0µg/dl on diastolic blood pressure was1.03 (95%CI: 1.01-1.05)with p=0.05**. Conclusion:** This study showed an association of high BLL with diastolic blood pressure but not with TG, FBS, and HDL-C, LDL-C and CHOL .This result persisted even after adjustment was made for age, BMI and job experience , smoking and eating habits. Attention to health-protective policies, individual behavioral changes and regular periodic medical examination with focusing on diastolic blood pressure in lead exposed workers is likely to decrease the substantial public health burden of ischemic heart disease.

Keywords: lead, hypertension, hyperlipidemia, hyperglycemia, ischemic heart disease

FOLATE, VITAMIN B12, AND HOMOCYSTEINE STATUS IN RAYONG PROVINCE; MAP TA PHUT AND PLUAK DAENG, THAILAND

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This cross-sectional study investigated effect of serum folic acid (FA) and serum vitamin B12 level on hyperhomocysteinemia in population who lived in Map Ta Phut and Pluak Daeng, Rayong province. The total of 200 subjects (35 males and 165 females) consisted of subjects lived in Ban Plong community where located around 3 kilometers from Map Ta Phut industrial estate, while Pluak Daeng subjects were selected from non-industrial locations. Fasting blood samples were collected and a questionnaire requested information on their lifestyle and potential risk factors (gender, age, and smoking habit etc.) were determined. The study showed that mean $\pm$ SD of plasma homocysteine (Hcy) levels in Map Ta Phut subjects were 15.4 $\pm$ 4.5 µmol/L. The hyperhomocysteinemia (>15 µmol/L) was 49 % of total subjects lived in Map Ta Phut and significantly difference with age ($P = 0.003$), serum vitamin B12 ($P = 0.047$), and FA ($P < 0.001$) while compared to normal serum level subjects. In male, mean of plasma Hcy (20.8 $\pm$ 5.2 µmol/L) were significantly higher ($P<0.001$) than females (14.4 $\pm$ 3.6 µmol/L). The mean FA and serum vitamin B12 levels were 11.7 $\pm$ 5.4 ng/mL and 694 $\pm$ 304 pg/mL, respectively that corresponding within the normal value (4.6 - 18.7 ng/mL;FA, 211-911 pg/mL;vitamin B12). The statistically significant ($P<0.001$) difference was shown between males and females for FA but were not significantly in serum vitamin B12. Mean plasma Hcy showed the level at 14.4 $\pm$ 4.1 µmol/L in Pluak Daeng subjects. The number of hyperhomocysteinemia was 34% of all subjects who were significantly with age ($P = 0.004$) and FA ($P = 0.008$) compared to normal serum level subjects. The plasma Hcy levels were statistically significant ($P < 0.001$) difference between males (19.0 $\pm$ 4.3 µmol/L) and females (13.3 $\pm$ 3.2 µmol/L). FA levels were 12.1 $\pm$ 4.5 ng/mL and serum vitamin B12 levels were 687 $\pm$ 275 pg/mL but there were not significantly between males and females. Our results conclude that hyperhomocysteinemia status was related with FA, and increasing age in both studied areas. Whereas, both vitamins; B12 and FA associated with plasma Hcy in Map Ta Phut. Thus, nutritional education for promoting food source of both vitamins to obtain their normal plasma Hcy status should be recommended.

INVESTIGATION OF LEAD AND CADMIUM IN ELEPHANTS' HAIR IN KANCHANABURI AND LAMPANG AREAS

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Although human hair analysis has been employed in forensic toxicology for several decades, the application in animals' hair as a tool to illustrate chronic exposure to drugs and environmental pollutants has recently gained much interest due to its practically convenience and painless sample collection procedure. The present study was designed to utilize the hair as the sample of choice to investigate the levels of cadmium (Cd) and lead (Pb) in captive elephants resided in two different areas in Thailand, Kanchanaburi and Lampang. Sixty-six animals were ranging between 4-59 years of age. Hair and blood samples were collected for metal analysis by ICP-MS and ICP-OES methods. Evaluation of animals' serum biochemical profiles and monitoring the metal levels in soil and water samples obtained from the 2 locations were also conducted. The results revealed that soil Cd level was significantly higher in the samples from Kanchanaburi, while the analysis of water samples from both sites showed no marked difference. The hair samples from Kanchanaburi elephants contained markedly greater level of Cd while the marked higher level of hair Pb was observed in samples collected from Lampang area. The results of biochemical analysis of serum samples from elephants between the 2 locations however exhibited no significant difference and the values were within normal ranges. In summary, elephant's hair could serve as a good sample of choice for metal analysis, which in turn could help indicate the environmental contaminant exposure.

Keywords: Elephant, Hair, Metal detection, Contaminated area

PREVENTION OF OCCUPATIONAL ACCIDENTS AMONG LABORATORY ANALYSTS USING RISK ASSESSMENT: A CASE STUDY ON NAFDAC

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Occupational risk assessment has been known to be an essential part of the planning stage of any health and safety management system in any organization. All workers are exposed to one form of hazards or the other. In the current study among laboratory analysts working in National Agency for Food and Drug Administration and Control (NAFDAC); a regulatory body in Nigeria, a semi-structured questionnaire was administered to all laboratory analysts in various sections of the laboratory. Personal interviews and observations were also made. Various hazards which include chemical, biological, physical and ergonomics hazards were identified with potential harms and possible victims. The assessment revealed that the employees and their visitors were exposed to these hazards. The existing control, both soft and hard measures were not adequate. Against this backdrop, risks among laboratory analysts should be reviewed from time to time with a view to remove all forms of hazards that could result into accidents.

STABILE DNA DAMAGE AND POLYMORPHISMS IN HOSPITAL WORKERS CHRONICALLY EXPOSED TO LOW DOSES OF IONISING RADIATION AFTER EXPOSURE TO 2 AND 4 GY GAMMA RADIATION

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The amount of DNA damage in hospital workers chronically exposed to ionising radiation (IR) varies among individuals and professions due to individual differences in IR response caused by inherited variations in DNA repair genes. The aim of the study was to examine wheter single nucleotide polymorphisms in selected DNA repair genes can predict DNA damage (micronuclei-CBMN assay) in peripheral lymphocytes of 56 hospital workers after exposure to 2 and 4 Gy. MN, NB and NPB showed significant difference between the nonirradiated and 2 Gy samples. MN, NB, NPB and NDI after 4 Gy significantly differed from unirradiated and from 2 Gy samples. After 2 Gy, proffessions of radiologists, ingeneers of medical radiology, anestesiologists and surgeons influenced on MN and polymorphic XPD rs 13181, hOGG1 and PARP1 variants had lower MN than homozygotes (HO), while polymorphic XRCC1, APE1 and XPD rs 1799793 variants had higher MN than HO. After 4 Gy, cardiologists, anestesiologists and surgeons showed positive correlation with frequency of nuclear buds, while radiologists showed negative correlation. Polymorphic XPD rs 13181, APE1 and MGMT C/T variants had higher frequency of nuclear buds than HO. Polymorphic XPD rs 1799793 and PARP1 variants had lower frequency of nuclear buds when compared with HO. An impact of profession and SNP polymorphism on the amount of DNA damage before and after irradiation in the exposed population showed that results can be useful in designing specific biomarkers for more susceptible subpopulation of people occupationally exposed to low doses of IR.

AMBIENT AIR QUALITY IN RURAL AND URBAN AREAS OF MYANMAR: AIR POLLUTION ASSESSMENT

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Baseline ambient air quality monitoring is the basis for the assessment of air pollution impacts generated by new development projects in the developing countries like Myanmar. This study was the first initiative air monitoring survey in Myanmar. The survey was aimed at revealing the existing ambient air quality prior to the potential air pollution impacts significantly affected by new economic developments. The cross-sectional survey had been carried out in the selected urban, sub urban and rural areas within Rakhine Coastal and Western Ranges Region, Central Lowlands Region and Eastern Highlands Region across the country during 2007-2011. Particulate matters and gases were detected using active air sampler with gas attachment, particulate monitor and diffusion tubes in accordance with the established guidelines. The baseline average levels of respirable PM_{10} and TSPM in rural area of Western Coastal region were 136±24.22 and 159±28.35 μg/m^3, Central Lowlands 110.33±22.38, 124.67±27.23 μg/m^3, Eastern Highlands 119.65±69, 132.33±12.41 μg/m^3 respectively. The PM_{10} and TSPM levels of major urban city in Lower Myanmar were 71.75±12.64, 143.21±27.37 μg/m^3 and Middle Myanmar 61.67±20.37 μg/m^3, 495.87±29.43 μg/m^3 respectively. Background fugitive dust levels in all selected regions did not meet the WHO air quality guidelines but were lower than National Ambient Air Quality Standards (USEPA). The emission sources for rural and urban mainly come from natural sources and mobile emissions respectively. Air quality for the SO_2, NOx and NO_2 in all regions was well below the guideline values. These findings can be applied as one of the step forwards to protect and mitigate the potential air pollution impacts on health and environment in Myanmar.

HEALTH CARE WASTE AND ITS POTENTIAL IMPACT TO HUMAN HEALTH IN KATHMANDU

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Health care wastes (HCW) are those wastes which are generated in the diagnosis, treatment or immunization of human beings or animals. These wastes are multi-hazard waste constituting infectious, radiological, chemical or physical hazards. The prevalent conditions of health waste management were studied among ten hospitals of the Kathmandu valley. A pre-designed questionnaire was used to take the data about the health care waste management in the respective hospitals and the composition of the waste was also analyzed. These hospitals were producing approximately 30% of infectious waste and 5 % of sharps. The data shows that HCW is not so managed properly by almost all the hospitals in the Kathmandu Valley and there is a belated for proper segregation of HCW. In some hospitals, they were try to segregate the wastes initially but mixed up among others during the final disposal. Moreover, they were only follow the simple burning or combustion of wastes including PVC materials in incinerator without controlled emission, and ultimately producing dioxins, NO_x, SO_x and the Hydrochloride gas. Thus, these may have significant health impact to the waste handlers and the public. About 13 mercury thermometers are disposed per day in the Kathmandu valley haphazardly, creating mercury pollution in air, water & soil and, ultimately affecting the biota including human beings. Positively, about 80 % hospitals were willing to pay for proper health care waste management.

Keywords: health care waste, dioxin, human health, hydroclave, autoclave

EXPOSURE OF VEGETABLE GROWERS DURING PESTICIDE APPLICATION

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In agriculture, pesticides are used to increase food quality, reduce crop loss and improve productivity. However, pesticides can also be hazardous if not used appropriately. In orer to evaluate the level of risks associated with pesticide application in vegetable growing zones, a study was carried out in Galim located between at 5^{o} $35^{'}$ -5 45 N, $10^{o}15^{'}$ -10^{o} 45^{1}E, 1000 masl (West Region, Cameroon) where farmers grow mostly tomatoes, peppers, huckleberry, cabbage, potatoes and watermelon. Interviews and fieldwork was carried out to know the type of pesticide used, spraying equipment, spraying attire and technical sta. The study revealed that vegetable growers in Galim were highly exposed to pesticides. This was shown by the high level of acute intoxications reported in the course of the study (61.7%) due to the use of large quantities o toxic pesticides (WHO class II), negligence in the use of pesticide protective measures (only 2.1 % of farmers use appropriate outfit for pesticide application), do not respect labels (76.6%) and consequently, do not know the risks involved in pesticide application. Vegetable growers are not trained (less than 22%) went to secondary school) neither are they followed up technically (only 8.5% of farmers can get information from ZEW) and consequently, 72.7% of farmers do not master pictogram. However, the understanding of pictograms did not necessarily reduce manipulation accidents. It is therefore important to sensitize farmers on the risks involved in pesticide application on human health, other living organisms, water, soil and air. Pesticide residue analysis is also needed to check the level of residue in the vegetables.

Keywords: Human health, Intoxications, Pesticide, Risk, Vegetable

RESIDUAL EFFECT OF THE PHYTOSANITORY TREATMENT OF ROUND LOGS ON THE SOIL OF THE WOOD PARK AT AUTHORITY PORT OF DOUALA

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This study on the some effects of phytosanitory treatment by chemical aspersion of logs at the wood Park of the Douala Port Authority; in the Littoral Region of Cameroon was carried out from April to September 2009. The principal objective was to the diagnosis the environmental effect of the phytosanitory treatment of logs before exportation. To conduct this study, two approaches were used: interview approach using questionnaire and sampling approach and soil analysis. For the interview approach, 150 questionnaires were administered to the personnel of the phytosanitory treatment company, to the phytosanitory controllers, to the operators of the wood section, to the guards company, to the managers of the wood park and also to the customers. This interview revealed that more than 70% of the interviewed persons have little knowledge on either the pesticide used in the treatment of wood in the park, or on the environmental impact of pesticide. For soil samples, the study zone was divided in six (06) blocks (A, B, C, D, E and F) of 1 hectare each. In each block, twenty (20) sub-samples were taken from which a composite sample was constituted to give a total of six (06) composite samples. The results of the analysis of the samples showed that 50% of the suspected active ingredient was found in the soil in identifiable residues. Some physico-chemical characteristic of the soil of the wood park influenced the fixation of molecule of the active ingredient. Actually, the results of the two approaches shows that the limited knowledge of disposition of phytosanitary treatment of logs inside the wood park and certain characteristic of the soil such as DT_{50}, Koc and Koe in this professional milieu had an effect on the residual effects of active ingredient of pesticides used for the protection of round logs. The reinforcement of phytosanitary control is then imperative in this zone of economic activities in view to assure environmental security while it is also important to quantify the residues identified.

Keywords: Log, Pesticides, Residues, Phytosanitary treatment

OCCUPATIONAL STRESS AMONG MIDWIVES IN PUBLC HOSPITAL

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Background: Job stress and health of personals of Health Care System has a great effect on their quality of patient care. Job stress and health of midwives as a part of this system regarding to the specifies of the job in care about two vulnerable groups of society: mothers and children. That is why the researcher decided to study on job stress of midwives in Public hospital of Tehran in 2004.**Methodology** : This research was a descriptive-analytic and cross-sectional study. Research setting was all Public hospitals of Tehran and data were collected by questionnaire and data. Sampling method was census like which 370 Midwives of public hospital participated in this research. Data were analyzed by descriptive (percentage, mean, standard deviation) and inference (Chi square and Fisher's exact tests) statistical method and by SPSS software. **Findings** : Results showed that job stress level of majority midwives (70.8) percent was normal and there was a meaningful statistical relation between job stress and academic degree (P = 0.032) and second earning resource (P = 0.011). Mental health of midwives (35.1%) was unsuitable and there was a meaningful statistical relation with academic degree (P = 0.001) and economic situation (P = 0.047). Meanwhile there was meaningful statistical relation between job stress and mental health (P = 0.001) of midwives. **Conclusion** : Although the majority of midwives (70.8) had normal job stress but (35.1%) of them had unsuitable mental health and regarding to need to mental health in provide suitable health care, that is suggested authorities with recognizing effective factors on job stress and state of health for using more from midwives' capabilities and providing facilities to consulting, welfare, sport and refractive lead to improvement state of health in midwifery personals.

Keywords : job stress, midwives, mental health, demographic characteristics

PROTECTION OF WORK HAZARDS AND PAINTING IN AUTOMOTIVE REPAIR SHOP, KHON KAEN MUNICIPALITY

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This descriptive survey study aimed to investigate the protection of painting hazard among automotive repair shops in Khon Kaen Municipality. There were 95 automobile repair shops in Khon Kaen Municipality where were permitted and operating. Data were collected by using the survey form and questionnaires. A total of 95 employers and 95 workers from each automobile repair shop by randomly sampling method were interviewed. Demographic characteristics, work accidents, work environmental factors, personal protective equipment (PPE) and annual medical check up were evaluated using questionnaires. The data were analyzed by conventional descriptive statistics: frequency, percentage, mean and standard deviation. The results showed that all of automobile repair shops located in a community. The workplace area was less than 400 square meters for 73 shops (76.84%) and the maximum area and the minimum area were 8,000 m^2 and 16 m^2, respectively. Most of shops were operated for ≤ 6 years (37.89%), followed by >6-13 years (27.37%), respectively. Shop was opened for 9 hours per day for 78.95% and shop was closed on Sunday for 91.58%. The average of repair service was for 1-3 cars per day (77.90%). There were 1-3 workers in the shop for 77.66%. The major automotive service was machine repair for 43.16%, followed by lubricator change for 27.37% and painting for 24.21%. Most shops had no specific booth for painting and used general area in the shop for 78.26%.The others had the booth for painting but there were no ventilation system or there were the systems without functioning. Most shops had no protection of painting spray spreading into atmosphere (86.96%). Among 95 workers, all workers were male, age was between 28-37 years for 40.01%, and work experience was less than 1 year for 52.63%. Most workers had no working suite for 86.32%. They used mostly cotton gloves for 54.47%. Workers had no personal protective equipment (PPE) for painting (86.96%) because of no support from their employers. Workers had not been medical checked up annual for 73.68%. The findings showed that the workers and nearby residences might be affected by exposure to chemical from painting in the automotive repair shop. There must be the painting booth available in the service shop included the ventilation system, the simple of waste and air treatment or painting process with the spray gun in order to protect chemical hazards in work environment. The employer should provide PPE and control of using PPE among workers and support workers the annual medical examination or at risk.

Keywords: Painting, Automotive Repair Shop, hazard, chemical

INTRA-CARDIAC THROMBUS FORMATION INDUCED BY CARBON MONOXIDE POISONING: A CASE REPORT

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Carbon monoxide (CO) is one of the leading causes of poisoning substances. It can inhibit the delivery of oxygen and cause sequential ischemic change, ending with death by multiorgan failure. And there are few cases about thromboembolic accidents by CO poisoning. However, intracardiac thrombus formation is rarely described as of yet. A previously healthy, 24-year-old woman was referred for carbon monoxide poisoning. She attempted suicide and her initial mental state was drowsiness with focal memory loss. Her initial carbon monoxide fraction was 16%. In the initial laboratory data, CK-MB 90.6 ng/mL (upper limit 5 ng/mL), Troponin I 1.9 ng/mL (upper limit 1.5 ng/mL) were observed. Transthoracic echocardiography (TTE) was done 24 hours from the accident. It revealed 30 x 15 mm nodular echogenic mass in the right atrium (RA). Anticoagulation with enoxaparin was started with hyperbaric oxygen therapy. After 7 days of heparinization, the large thrombus in RA disappeared. This report describes an intracardiac thrombus formation induced by CO poisoning. Because intracardiac thrombus can lead to pulmonary embolism and cerebral embolic infarction, the consideration of it in CO poisoning is important.

EPIDEMIOLOGIC CHARACTERIZATION OF POISONING BY ALDICARB IN RIO DE JANEIRO, BRAZIL

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Aldicarb is a systemic carbamate pesticide used to control nematodes in soil and insects and mites on a wide variety of crops, grain, including citrus fruits, potatoes, peanuts, soy beans, sugar-beet and tobacco. Nowadays, the commercialization of aldicarb has been controlled in several countries and others have been banned, but in Brazil this product may still be found. With his extensive use in Rio de Janeiro, Brazil, we can see a great number of human acute poisoning, a lot of these fatal, because the aldicarb has been illegally used (as rodenticide) since the 80's. Monthly arrive at the Forensic Institute about 4 suspected cases of poisoning by aldicarb. This study found that, in the period between 1999 and 2011, were received at the Laboratory of Forensic Toxicology 637 suspected cases of aldicarb poisoning, of which 79,9% were positive (509 cases). The contentious issue is of great relevance to public health and aims to draw the attention of government authorities so that this pesticide is finally banned in our country.

PSP (PARALYTIC SHELLFISH POISONING) TOXINS IN PHYTOPLANKTON OF THE GULF OF THAILAND

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The occurrence of PSP toxins (as saxitoxins equivalent) was investigated from 387 phytoplankton samples representing 21 localities in 5 regions of the Gulf of Thailand. Receptor Binding Assay (RBA) demonstrated PSP toxicity in 19 localities (86 samples). The total toxin contents varied between 13.3 and 1387.7 with the median of 92.5 ng STX equivalents L^{-1} SW. Two samples from 2 localities contained more than 1000 ng STX equivalents L^{-1} SW (1032.2 and 1387.7 ng STX equivalents L^{-1} SW, respectively). Both two located in the Upper Gulf of Thailand with PSP constituting more than 70% of the total toxin content. In contrast, the highest frequency of phytoplankton found PSP positive (40% of 43 samples) were in the Andaman Sea. The second most frequent found were in the Eastern Gulf (28% of 86 samples); followed by the Upper Gulf (20% of 194 samples), Southern Gulf (15% of 34 samples) and Central Gulf (6% of 30 samples), respectively. Temporal variations showed no significant correlation between toxin contents and seawater physical and chemical properties i.e. dissolved oxygen, pH, salinity, temperature, and transparency. Toxin contents peaked in dry season from February to April then increased again but at a lesser extent in July to August. PSP concentrations in toxin test positive phytoplankton showed no correlation with those in bivalves (from our previously investigation) with the correlation coefficients R^2=0.001.

TOXICOLOGY FINDINGS OF BLOOD METHAMPHETAMINE CONCENTRATIONS AND MANNER OF DEATH IN BANGKOK

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Methamphetamine (MA) is the major illicit drug in Thailand. The blood MA concentrations represent the symptomatic of MA appearance and intoxication such as hallucination, abnormal excitement, poisoning and finally death. Seventy MA-related deaths out of total of 2,018 unnatural autopsy cases (3.46%) of Institute of Forensic Medicine were collected from April to August 2011. The concentrations of MA in blood were analyzed by GC/MS/MS Triple Quadrupole (Agilent 7000B GCMS Triple Quadrupole, USA). In this study, the mean age for 67 males and 3 females was 32.3 years. Mean blood MA concentration was 12.8 ug/mL (range from 59 ng/mL to 717 ug/mL). The high concentrations of blood MA over 1500 ng/mL (24 of 70 cases) were found in manner of death which due to violence and crime (homicide 41.7%, suicide 8.3% and accident 16.7%). On the other hand, 33.3% of undetermined group occurred as a consequence of MA-related death. These findings indicated the importance of social problems due to MA abuse and MA-related death in Bangkok.

A CASE OF METHEMOGLOBINEMIA AFTER INGESTION OF NAPROXEN

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To report a case of acute methemoglobinemia in a patient treated with naproxen for the common cold. A 42-year-old asian woman received atenolol 25mg and atorvastatin 20mg for 6 years for hypertension and hyperlipidemia; lorazepam 1mg, trazodon 50mg, and paroxetine 20mg for 3 years for alcohol dependence and depression from a local psychiatric clinic, and acamprosate 333mg for 6 months for alcohol dependence. Yet she continued to ingest alcohol (20%) 300~600mL every day. Three days prior to visiting the ER, the patient began taking Naproxen sodium and Methocarbamol for mylagia, chills and coughing. One day prior to visiting the ER, the patient was presently taking lorazepam, trazodon, paroxetine in addition to the Naproxen and Methocarbamol prescribed for the cold symptoms and ingested approximately 300mL of alcohol (20%) and then went to sleep. She soon developed dyspnea and dizziness. She was found to have severe methemoglobinemia (serum methemoglobin fraction 49%; reference range 0~0.2). Her symptoms improved substantially, and serum methemoglobin levels decreased after the initiation of methylene blue therapy. 10 days after visiting the ER, she was discharged without any complications. Currently there has been no report of Naproxen intake causing methemoglobinemia. However, Naproxen is known to cause oxidative stress. In particular, in G6PD deficiency, oxidative stress has been reported to cause hemolysis or methemoglobinemia. Furthermore, alcohol is known to cause G6PD deficiency and thus it is hypothesized that the administration of naproxen in an alcohol dependent patient caused methemoglobinemia. Care must be taken when prescribing Naproxen to an alcohol dependent patient.

ASSOCIATION BETWEEN *P53* CODON 72 POLYMORPHISM AND GASTRIC CANCER RISK IN THAI POPULATION

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Gastric cancer is a new public health problem in Thailand since the incidence and mortality rate of this cancer are increasing yearly. *P53* codon 72 polymorphism has been reported to be associated with various cancers including gastric cancer. The objective of this study was to evaluate an association between *P53* codon 72 polymorphism and gastric cancer risk in Thai population. The hospital based case-control study was conducted in 96 gastric cancer patients and 148 healthy controls (age-matched) by using real-time polymerase chain reaction with a TaqMan probe assay. The frequencies of Arg/Arg, Arg/Pro and Pro/Pro genotypes of the *P53* codon 72 polymorphism were 33.8 (50/148), 47.3 (70/148), and 18.9 (28/148) % in the controls and 22.9 (22/96), 50.0 (48/96) and 27.1 (26/96) % in the gastric cancer patients, respectively. The Pro/Pro genotype was associated with an increased risk for gastric cancer development [odds ratio = 2.11, 95% confidence interval (CI) = 1.01-4.39, P = 0.04]. However, the significant interactions between the *P53* codon 72 polymorphism and smoking and drinking were not observed. The results of this study suggest that *P53* codon 72 polymorphism is associated with gastric cancer risk and the Pro/Pro genotype may be a useful risk factor for prediction of gastric cancer development in Thai population.

MULTIPLE ORGAN FAILURE AFTER OXALIC ACID INGESTION

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INTRODUCTION: Oxalic acid ingestion, available as laundry bleach, is an emerging agent among self-poisoning cases referred to the poison center with a high case fatality. CASE PRESENTATION: This is a case of 28 year old man who presented with systemic complications after intentional oxalic acid ingestion. Patient was seen with abdominal pain following ingestion of 10 g oxalic acid mixed with water. Caustic injury was ruled out normal endoscopic findings. Acute kidney injury evidenced by acute oliguric renal failure ensued within 6 hours. Patient underwent five sessions of hemodialysis and before renal function indices returned to normal and calcium oxalate in the urine was no longer evident. Other systemic manifestions noted were metabolic acidosis with elevated anion gap with persistent hypocalcemia, and episodes of QT prolongation. Respiratory distress developed on the third day with signs of acute lung injury. Supportive management was given with hemodialysis, Supportive mechanical ventilation, hydration, and electrolyte correction were given. Use of antidotes such as N-Acetylcysteine as nebulization to address lung injury and potassium citrate postulated to inhibit calcium oxalate supersaturation in the renal tubules were included in the treatment regimen to address organ specific injury from oxalate. Patient was discharged after intensive care. DISCUSSION: Pathophysiologic findings following oxalic acid ingestion results from intraluminar crystal deposition with renal biopsy specimen showing the degeneration of the renal tubular epithelial cells associated with intracellular calcium oxalate crystal deposition resulting to acute renal failure. Systemic deposition resulting to widespread oxalosis, is postulated to cause the multiorgan failure exhibited in the course of the patient. LESSONS LEARNED: Early identification of systemic complications of a caustic substance is imperative with adequate supportive management to avoid morbidity. It is recommended that oxalic acid ingestion be included in the continued toxicovigilance and promotion of regulation controls on availability of toxic household chemicals be implemented.

Keywords: Oxalic acid ingestion, Acute renal failure, Household chemical

MERCURY INDUCED OXIDATIVE STRESS AND THE ROLE OF N-ACETYL CYSTEINE AND SELENIUM SUPPLEMENTATION DURING CHELATION: A POSSIBLE APPROACH

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Mercury toxicity is also considered the second-most common cause of acute heavy metal poisoning, with 3,596 cases reported in 1997 by the American Association of Poison Control Centers. Mercury and its derivatives have become an alarming environmental problem, necessitating the search for effective companion formula, which are generally used in micro doses and are devoid of any palpable side-effects. This study was undertaken to study the protective effect of N-acetyl cysteine and selenium alone or in combination against mercuric chloride toxicity in rats. Male *Sprague Dawley* albino rats (150 ± 10 g) were randomly divided into five groups. Group 1 served as control. Group 2-4 were administered $HgCl_2$ (12 µmol/kg, *i.p.*) once only and group 2 served as experimental control. Animals of group 3, 4 and 5 received NAC (NAC: 2 mM/kg, *i.p.*), Se (Se: 0.5 mg/kg, *p.o.*) and NAC + Se. Compared to the control, $HgCl_2$ treatment provoked significant increase ($P \leq 0.05$) in of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), bilirubin, γ-glutamyl transpeptidase (γ-GT), cholesterol, triglycerides (TG), protein, urea, creatinine, uric acid, blood urea nitrogen (BUN) content with a decrease albumin concentration in serum. The results demonstrate that treatment with NAC, Se and NAC+ Se provided protection against mercuric chloride treatment caused the altered indices to return to near normal levels. Histopathological analysis was consistent with the biochemical observations and led to conclude that combination therapy provided protection against mercury toxicity.

Keywords: Mercury, N Acetyl Cysteine, Selenium, Oxidative stress

ANTIOXIDANTS WITH CHELATING AGENTS IN THE DIAGNOSIS AND TREATMENT OF PUTATIVE MERCURY POISONING

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Mercury commonly used in industrialized countries, adversely affects human and animal's physiological and biochemical functions. Mercury exposure is the second-most common cause of toxic metal poisoning. Male albino rats were administered a bolus dose of dimethylmercury (1.0 mg/kg) orally for 12 weeks (5 days/week). The chelation therapy with NAC alone and combination with antioxidants as zinc and selenium was given for (2 days/week) after toxicant administration. Animals of all groups were sacrificed after 48 h of last treatments and various blood & biochemical parameters were performed. In the present study demonstrated that chronic exposure of dimethylmercury led to mark a significant rise in bilirubin, γ-GT, cholesterol, triglycerides, urea, creatinine and uric acid content with a concomitant decline in albumin concentration. Significant elevation was observed in LPO and mercury concentration in liver, kidney & brain however a concomitant decline was observed in GSH level after toxicant administration in the same organs. A noticeable fall was observed in the brain marker enzyme acetyl cholinesterase. Combined treatment of zinc and selenium with N-acetyl cysteine to dimethylmercury-exposed rats showed a substantial reduction in the levels of DMM-induced oxidative damage and comet tail length. In conclusion, the results of this study support that the supplementation of zinc and selenium with N-acetyl cysteine can improve theDMMinduced blood and tissue biochemical oxidative stress and molecular alterations by recoupment in mean DNA damage.

Keywords: N-acetyl cysteine, zinc, selenium, lipid peroxidation, reduced glutathione, acetyl cholinesterase

HEMATURIA, AN UNUSUAL SYSTEMIC TOXICITY, IN FORMIC ACID INGESTION: A CASE REPORT

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Systemic toxicities occur in some caustic ingestion patients, however hematuria is a rare toxicity reported. In this report, we describe a case of a 1-year-old female child who accidentally ingested liquid formic acid. This patient had severe local corrosive effect included upper respiratory tract compromise and developed gross hematuria during the hospital admission and resolved without specific treatments. The investigations worked up for the cause of gross hematuria were done but could not find any abnormalities. Hematuria might be one of the systemic effect from caustic ingestion.

JUGLANS REGIA L. (WALNUT) EXTRACT AMELIORATES CYCLOPHOSPHAMIDE INDUCED ACUTE LUNG INJURY IN MALE RATS

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Cyclophosphamide (CP) is an alkylating antineoplastic drug widely used in cancer chemotherapy. Among the various side effects of CP, lung toxicity is a major deterrent in its clinical use. *Juglans regia L.* commonly known as walnut has been proven as a good protective agent against pulmonary toxicity because it is rich in antioxidants content. We wanted to study the prophylactic effect of total methanolic extract of *J. regia* on the toxicity profile of CP with special focus on pulmonary toxicity. An acute study was conducted on male Wistar rats. In a 7 day of treatment schedule, rats were exposed to CP (250 mg/kg b.wt. *i.p.*) and plant extract (200mg/kg b.wt. *per os*) Antioxidant profile in lung tissue and enhancement in the level of inflammatory markers in the bronchoalveolar lavage fluid (BALF) was measured along with histopathological examination. A marked decrease in the activities of antioxidant enzymes such as glutathione peroxidase, glutathione reductase, glutathione *S*-transferase, superoxide dismutase and catalase were observed in the lung tissue which was significantly restored by *J. regia* extract. *J. regia* extract significantly decreases the protein carbonyl content which was significantly increased by CP indicating protein oxidation. Treatment of *J. regia* also caused significant decrease in lipid peroxidation and increase in reduced glutathione content in rat lungs. In BALF, a marked enhancement in the level of inflammatory markers such as alkaline phosphatase, lactate dehydrogenase, myeloperoxidase and total cell count was observed which was diminished by *J. regia.* Histological findings strongly support these biochemical observations. Histological examination of lungs showed changes such as inflammation and fibrosis in CP exposed rat lungs which was attenuated by plant extract. CP exposure to rat lungs showed a toxic effect, especially disruption of lung antioxidant profile and inflammatory response in BALF. *J.regia* proved as a powerful protective agent against CP induced acute lung toxicity in rat lungs.

POISON CENTRE'S IN AFRICA: A ONE YEAR COMPARATIVE STUDY (2011)

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Aim: According to the World Health Organization there are currently 14 Poison Information Centre's in Africa. The aim of this pilot study was to comparing the spectrum of consultations dealt with by African Poison Centre's. **Methods**: Questionnaires were sent to Centre's. Information required: number and time of communications, interlocutors, patient demographics, intentional/accidental, substance categories, substances most commonly encountered. **Results**: 44% responded. Two centre's use the INTOX Database provided by WHO. Kenya and Zimbabwe provided estimations of communications. Morocco received the largest number of communications (36681) of which 57% were by mail. South Africa 6232, Kenya 469 and Zimbabwe – (%estimation due to incomplete recording) all received only telephonic consultations. 92% of Moroccan calls were received within office hours, other centre's calls varied between 10h00 and 22h00. Age distribution was similar (±50% vs ±50%) except in Zimbabwe where 82% were due to adult exposures. 82% of exposures in Morocco were accidental whereas when compared to the other centre's the ratio of accidental to intentional were ±58% vs ±42%. South Africa and Morocco dealt with similar percentages of exposures in children and adults (52% vs 48%). 82% of exposures dealt with in Zimbabwe were adults. Pharmaceutical exposures varied between 7% to 45%, non-drug chemicals between 15% and 69% and biologicals between 8% and 75%. 74% of cases in Morocco were due to scorpion sting, pesticide exposures were highest in Kenya (42%) and Zimbabwe (36%). In South Africa analgesics, e.g. paracetamol, was responsible for most exposures. **Conclusion**: An important finding was the lack of reliable data collection. Morocco receives most communications by mail. An interesting observation was the high number of scorpion sting dealt with by the Moroccan Centre. The other Centre's deal mostly with pesticide exposures. Further analysis of comprehensive PIC data is needed before a meaningful conclusion can be made.

EFFECTS OF LAMOTRIGINE, LEVETIRACETAM AND TOPIRAMATE ON SERUM TRACE ELEMENT STATUS AND OXIDATIVE STRESS IN RATS

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Background and Objectives: Experimental and clinical studies indicate that conventional antiepileptic drugs (AEDs) can alter the homeostasis of trace elements, electrolytes and increase membrane lipid peroxidation at the expense of protective antioxidants. This information is lacking for the newer AEDs. We therefore studied trace metal status and oxidative stress in rats treated chronically with, lamotrigine, levetiracetam and topiratmate in comparison with valproate. **Methods:** Thirty male wistar rats were randomly divided into 5 groups and treated orally with vehicle (controls), sodium valproate (370 mg/kg), lamotrigine (50 mg/kg), levetiracetam (310 mg/kg) and topiramate (100 mg/kg) for 45 days. The level of serum zinc, selenium, copper, iron, manganese, cobalt and aluminium were determined using inductively coupled plasma-atomic emission spectrometry(ICP-AES) at baseline and at the end of treatment. Oxidative stress parameters [malondialdehyde (MDA), reduced glutathione (GSH) and superoxide dismutase(SOD)] were also estimated in the rat brain. **Results:** Serum iron and cobalt levels were significantly reduced with all the three newer AEDs ($P < 0.05$) while copper levels were reduced in the rats treated with valproate ($P < 0.05$) and levetiracetam ($P < 0.01$). Animals treated with levetiracetam had significantly increased aluminium levels ($P < 0.05$) while zinc levels were reduced ($P < 0.01$). Serum selenium levels were reduced in the valproate, levetiracetam and topiramate treated groups. In comparison to control group, MDA was higher in the levetiracetam ($P < 0.01$) and topiramate ($P < 0.05$) groups. GSH and SOD activity were significantly reduced by valproate, levetiracetam and topiramate treatment. **Conclusion:** Treatment with dose equivalent to maximal therapeutic doses of AEDs can produce significant alterations in the trace element status. Reduced levels of iron, cobalt and copper may be responsible for some of the hematologic adverse effects of newer AEDs while reduced selenium levels may be associated with increased oxidative stress as seen with valproate, levetiracetam and topiramate in this study.

BLADDER CANCER DOCUMENTATION OF CAUSES: MULTILINGUAL QUESTIONNAIRE "BLADDER CANCER DOC"

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There is a considerable discrepancy between the number of identified occupational-related bladder cancer cases and the estimated numbers particularly in emerging nations or less developed countries where suitable approaches are less or even not known. Thus, within a project of the World Health Organisation Collaborating Centres in Occupational Health, a questionnaire of the Dortmund group, which has been applied in different studies, was translated into more than 30 languages (Afrikaans, Arabic, Bengali, Chinese, Czech, English, Finnish, French, Georgian, German, Greek, Hindi, Hungarian, Indonesian, Italian, Japanese, Kannada, Kazakh, Kirghiz, Korean, Malay, Persian (Farsi), Polish, Portuguese, Portuguese/Brazilian, Romanian, Russian, Serbo-Croatian, Slovak, Spanish, Spanish/Mexican, Tamil, Telugu, Thai, Turkish, Urdu, Vietnamese). The bipartite questionnaire asks for relevant medical information in the physician's part and for the occupational history since leaving school in the patient's part. Furthermore, this questionnaire is asking for intensity and frequency of certain occupational and non-occupational risk factors. The literature regarding occupations like painter, hairdresser or miner and exposures like carcinogenic aromatic amines, azo dyes, or combustion products is highlighted.

ASSOCIATION BETWEEN HLA GENETIC POLYMORPHISM AND PHENYTOIN-INDUCED SEVERE CUTANEOUS ADVERSE DRUG REACTIONS IN A THAI POPULATION

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Aromatic antiepileptic drugs (AEDs) including carbamazepine (CBZ), phenytoin (PHT) and phenobarbital (PNB) are the common culprit drugs for severe cutaneous adverse drug reactions including Stevens–Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN). Cross hypersensitivity between these AEDs, particularly between CBZ and PHT, have been reported. Recent evidences suggest that *HLA-B*15:02* allele associate with the CBZ-induced SJS/TEN in certain ethnics. Although association between *HLA-B*15:02* and PHT-induced SJS/TEN have been demonstrated in Han Chinese and Thai patients, the number of patients enrolled in those studies were very small. The aim of the present study was to determine the degree of association between PHT-induced SJS/TEN and *HLA-B*15:02* as well as to explore the association between other HLA allele and PHT-induced SJS/TEN. Twenty five PHT-induced SJS/TEN and 74 PHT-tolerant patients were recruited in this study. Genomic DNA of each patient was extracted from peripheral blood. The HLA genotypes were determined using specific sequence oligonucleotide method. We found that the heterozygous *HLA-B*15:02* was present in only 20% (5/25) of PHT-induced SJS/TEN patients whereas 14.86% (11/74) of PHT-tolerant patients carried this allele. There was no statistical significant association between *HLA-B*15:02* allele and PHT-induced SJS/TEN which conflicts with previous studies. However, PHT-induced SJS/TEN were statistical significant associated with *HLA-B*51:01, HLA-B*56:04* and *HLA-C *14:02*. In conclusion, *HLA-B* 51:01, HLA-B*56:04* and *HLA-C *14:02* but not the *HLA-B*15:02* allele may be a good genetic marker for PHT-induced SCAR in a Thai population. However, due to limited number of patients enrolled in this study, these association warrant to be confirmed in larger population.

HEALTH RISK ASSESSMENT ON THE CONSUMPTION OF LEAD-CONTAMINATED AQUATIC ANIMALS FROM FISHERY RESOURCE IN THE OVERFLOW MARSH

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This cross-sectional survey research aimed to determine lead exposure and risk assessment on the consumption of lead-contaminated aquatic animals from fishery resource in the overflow marsh. The interviews of 75 residents at Loeng Puay on the consumption of the aquatic animals and vegetable (n=75) were conducted. The lead concentrations were analyzed by using Atomic Absorption spectrophotometry (AAS). The lead concentrations in Nile tilapia (n=20), Common silver barb (n=20), River snail (n=20), Golden apple snail (n=20), Morning glory (n=20) and Kale (n=20) were between 0.02-0.07, 0.01-0.22, 0.36-0.28 and not detected -0.95 mg/kg dry weight, respectively. The concentration in the most aquatic animals were not exceeded the standards, except the lead concentrations of River snail which were exceeded the Thailand standard as defined in food (1 mg/kg) for 30.00% and exceeded the Australia and New Zealand standard as defined in molluscs (2.0 mg/kg) for 10.00%. Health risk assessment on the consumption of lead-contaminated aquatic animals was performed according to the US.EPA guideline (2004) at total intake rate of aquatic animals which was at 95th percentile 307.26 g/day. Health risk assessment on the consumption of lead-contaminated aquatic animals showed that the lifetime average potential dose of lead ingestion aquatic animals from this marsh was 10.58 μg/kg/day. The exposure level was not exceeded Provisional tolerable weekly intake (PTWI) for lead (25 μg/kg/day by JECFA, 1993) or no risk. However, the potential health risk from lead exposure depending on an individual intake rate and type of aquatic animal, particularly ingestion together with other kinds of lead contaminated foods. Therefore, there should be the surveillance program by environmental monitoring in the fishery resource where is the overflow marsh receiving treated water and waste water from community and agriculture.

THE OPTIMIZATION OF REAL-TIME QUANTITATIVE PCR USING SYBR GREEN FOR DETECTION OF AFLATOXININ MAIZE SAMPLE FEEDSTUFFS

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Aflatoxins are produced by Aspergillus species growing in feedstuffs. A *aflR* gene is involved in regulation of aflatoxin biosynthesis and sequence has been published. The detection of early contamination of maize by aflatoxigenic should be useful, becauseaflatoxins have important health effects. In the present study, a quantitative real-time PCR (qPCR) assay was optimized for detection and quantification of aflatoxin in maize, detection amplified template was accomplishedwith SYBR Greenwas developed. DNA extractions were performed from thirty- five maize samples, and subjected to real-time PCR. The developed real time PCR system was also used to analyze the occurrence ofaflatoxinproducing on maize. Reference strains with a PCR protocolbased on the fungal B-tubulin gene, which was used asapositive fungal control. This qPCR protocol should be the choice of interest, on the determination of the aflatoxincontent in maize samples feedstuffs.

Keywords : Aflatoxin, A. flavus, real-time PCR, SYBR Green, Maize, Feedstuffs

DIETARY EXPOSURE ASSESSMENT OF BENZOIC ACID IN THAI POPULATION ESTIMATED FROM THE CODEX FOOD STANDARD

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The Thai Food and Drug Administration was in the process of revising the food additive regulations to follow Codex General Standard for Food Additives (GSFA). The maximum levels (MLs) of food additives used in foods, established in GSFA were assessed regarding the risk on consumer health based on dietary exposure in Thai population to ensure safety. Benzoic acid is a popular food preservative used in many food categories in Thailand and many countries. Thus, there is a need to estimate the risk from dietary exposure in order to develop the regulation that conforms to international standards and ensures national food safety. This study aimed to assess the dietary exposure of benzoic acid in Thai population based on the MLs proposed in GSFA. The exposure was estimated by combining food consumption data with the data on the MLs of benzoic acid in food given in GSFA Rev 8-2007. Thai food consumption data were estimated through food consumption surveys at an individual level in 2006. Probabilistic exposure assessment (Monte Carlo simulation) was conducted to estimate the degree of exposure at the average exposure and the high exposure (97.5^{th} percentile). Risk was characterized by exposure beyond the Acceptable Daily Intake (ADI) of benzoic acid advised by the Joint FAO/WHO Expert Committee on Food Additives. The study found that the average benzoic acid exposure in general population aged up to 3 years exceeded the ADI (126% of ADI). The population aged 3-5.9 years and 6-18.9 years had the exposure levels higher than the other aged groups at 226 and 153% of ADI, respectively. High exposures were found in non alcoholic beverages, bakery wares, processed fruits and vegetables. It indicates that there is a risk concern especially in children when using benzoic acid in food according to GSFA. Thus, the MLs of benzoic acid proposed in the Codex food standard should be reduced in some food categories based on food safety risk assessment and technological justification.

Keywords: Benzoic acid, Exposure assessment, Codex food standard

Research funded by the Thai Food and Drug Administration

THE POTENTIAL CHEMICAL HAZARD IN THE PRODUCTION OF FRESH AND DRIED RED CHILI

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Inadequate awareness on agricultural chemicals applied and not enough precaution on production, harvest and storage of red chili may pose serious chemical hazards. The objective of this study was to investigate the possibility of potential chemical hazards occurring in fresh and dried red chili production chain. Chili production during cultivation and post harvest handing were studied comparative between 10 good agricultural practices certificated farms and 10 conventional farms in order to assess the potential hazard entering in these activities and unsuitable practices that are possible cause of contamination. It has revealed that pesticide is the serious chemical hazard in fresh chili produces due to misuse during cultivation including incorrect withholding period and mixing-over dose. A risk level of pesticide was conducted using risk ranking that uses matrix has rang of likelihood of pesticide residue on product and consequences of pesticide toxicity on human health axes. It was found Cypermethrin, EPN and Frometanate were pesticides of most concern with the requirements of closely surveillance. Improper post harvest handling was drying material on dirty surface, never be covered by clean gunny bags at night and stored with no protection from dampness posing the contamination of mycotoxins. Twelve dried red chili samples obtained from farm and warehouse were found aflatoxin levels in 4 (33%) of 12 samples ranging from 2.55 to 67.45 ppb. One sample had level higher than the maximum tolerable limit (20 ppb), according to the Ministry of Public Health No. 98 (BE 2529) in Thailand. Maximum AFB1 level was 64.58 ppb in dried chili collected from warehouse. However, aflatoxin contamination levels could be decreased if taken on hygiene control. Aflatoxin levels of dried chili samples purchase from the market were slightly decreased after treatment with suitable cleaning and drying conditions. The results in this study indicate that fresh chili is at especial risk for pesticide contamination by incorrectly applied and unsuitable post harvest handling caused hazard of aflatoxin contamination in dried chili. Improper production practices are more found in conventional farms than GAP certificated farms.

Keywords: Pesticide, Aflatoxin, Red Chili

Research funded by National Bureau of Agricultural Commodity and Food Standards

RELATIONSHIP BETWEEN LOW-DOSE BISPHENOL A AND BENIGN HYPERPLASIA PROSTATE

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Benign prostatic hyperplasia (BPH) is a common condition in men over 50 years of old. Within physiologic range, increasing estrogen levels can stimulate prostate to develop and permanently increase prostate size. As an estrogenic endocrine disruptor, low-dose bisphenol A (BPA) might be stimulatory to prostate development. We further hypothesized that low-dose BPA could induce hyperplasia prostate to proliferate and aggravates symptom of BPH in rats and old dogs. Thus, BPH model of rats induced by testosterone were treated with BPA (10-90μg/kg, i.g.,daily) for 4 weeks, adult rats were treated with BPA (10-90μg/kg, i.g., daily) for 4 weeks, and spontaneous BPH model of old dogs were treated with BPA (2-18μg/kg, p.o., daily) for 2 months. In BPH rats, we found that BPA significantly increased the weight of prostate ($P<0.01$), especially for the weight of dorsolateral prostate (DLP), and increased the height of epithelial cell(HEC) of DLP. In adult rats, BPA increased weight, volume and relative weight of prostate, especially significantly increased the weight of DLP ($P<0.01$), and also increased HEC of DLP ($P<0.01$). While in old dogs, BPA (2μg/kg/day) significantly increased prostate weight ($P<0.05$), prostate volume ($P<0.05$), and HEC of prostate ($P<0.01$). We concluded that environment exposure to low dose of BPA could increase the prostate size of adult rats, aggravate testosterone-induced BPH in rats and spontaneous BPH in old dogs, these results suggest that low dose BPA may have the same effects on the prostate of human being, but this need further study to validate.

Keywords: Bisphenol A, prostate, BPH, rat, Old dog, Low dose, Endocrine disruptor

SAFETY ASSESSMENT OF COSMETIC PRODUCTS: REFINING EXISTING AND INTEGRATING NEW APPROACHES

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Risk assessment is essential to assure the consumer safety of cosmetic products, before they are placed on the market. This requires a thorough review of the available scientific evidence of toxicological hazard – the potential to cause harm – together with a realistic assessment of consumer exposure to the product. It is essential that all relevant toxicological endpoints are addressed, focusing on the individual constituent ingredients and any gaps or uncertainties in the data. Characterisation of toxicological hazard has often relied upon established animal models. Unilever has a longstanding commitment to the development and use of alternatives to animal tests; these *in vitro* assays and *in silico* modeling/predictions must be carefully interpreted alongside pre-existing data in a meaningful risk assessment. Understanding consumer exposure is equally important for a robust risk assessment. This goes beyond simply looking at the level present in the product formulation; requiring thorough understanding of how the product is intended to be used, or indeed potentially misused. It is important to consider all relevant routes of exposure, the area and duration of skin exposure, the ability of the various ingredients to penetrate the skin, and whether the product is left on or rinsed off (and, if rinsed, how much of the ingredient would be retained in the residue). It is also important to tailor the exposure assessment to the target organs or tissues relevant to the hazard; bodyweight adjusted systemic dose is relevant for systemic toxicity, whereas dose per unit area of skin is relevant for skin sensitisation. In some cases it may be necessary to consider whether specific subpopulations are at greater risk, either on the basis of a particular susceptibility to the hazard, or increased exposure due to physiology or behaviour. Thorough risk assessment of ingredients and formulations means that consumers can use cosmetic products with confidence.

HEALTH RISK ASSESSMENT OF DAILY NITRITE AND NITRATE INTAKES FROM PROCESSED MEAT AND FISH PRODUCTS IN THAI SCHOOL CHILDREN

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Nitrite and nitrate are commonly used as food additives in processed meat and fish products because of a variety of technical functions. The acceptable daily intakes (ADI) of nitrite and nitrate, the amount of the additive that can be ingested daily through a lifetime without any adverse effect, were established at low levels. Thus, it is important to estimate the health risk of nitrite and nitrate exposures from the consumption of processed meat and fish products among a high-risk subgroup that is school children and identify the high-risk products of concern. In this study, the 'risk' was characterized by comparing the exposure to the ADI in 2 different scenarios. The first scenario, the exposures to nitrite and nitrate were assessed in 7,096 Thai school-age children based on national food consumption data (food frequency questionnaires) in 2003 and the concentration data of processed meat and fish products from 5 regions of Thailand. The second scenario was conducted in Nakhon Pathom province by collecting the consumption data (24 h recalls) from 432 students in 2011 and subsequently collecting samples from the school areas. The results of both scenarios showed that the school children exposed to nitrite from a whole processed meat and fish greater than nitrate, but did not exceed the ADI. Young children exposed to high levels of nitrite (19% of ADI for children aged 6-9 lived in 5 regions and 12% of ADI for Nakhon Pathom students aged 7-12). Sausage was a major contributor to nitrite intake because of most common high concentration. The maximum use level for nitrite permitted in cured meats including sausage was 125 mg $NaNO_2$/kg. However, high consumption (97.5^{th} percentile) of sausage which contained the highest nitrite content (99 mg $NaNO_2$/kg) provided exposure to nitrite exceeded the ADI in Nakhon Pathom students. Thus Thai FDA should to revise the maximum level of use for nitrite in this product and recommend safe to eat for children.

Keywords: Nitrite, Nitrate, Processed meat and fish, School children, Risk assessment

DIETARY EXPOSURE TO BENZOIC ACID AND SORBIC ACID FROM NON-ALCHOLIC BEVERAGES CONSUMPTION OF THAI STUDENT

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Benzoic acid and sorbic acid are food preservatives, allowed to use in non-alcoholic beverages because they have low toxicity to human. However, high intake of benzoic acid and sorbic acid may pose adverse health affect for consumer, especially for children who consumed higher amount of non-alcoholic beverage than adult when expressed on a bodyweight. This study aims to assess the health-risk related to benzoic acid and sorbic acid exposures from non-alcoholic beverages in Thai students. The exposures have been calculated from the data of food consumption and concentration. Two different food consumption databases were used in this study. The first database was collected from 7,096 students at all regions (17 provinces) in 2004, using food frequency questionnaire. The second database collected from 432 students in middle region (Nakhon Pathom province) in 2011, using 24 hours recall. The non alcoholic beverages (367 samples) were collected from all regions (16 provinces) and determined for benzoic acid and sorbic acid contents using high performance liquid chromatographic method. The results found that the dietary exposure to benzoic and sorbic acid via beverage consumption in student from both databases was lower than the ADI (The health based guidance value). Furthermore, the dietary exposure estimated from the second database was higher than the first because such beverages consumption data was collected in 2011, which found two times increasing over the consumption collected in 2004. Although the exposures did not exceed the ADI, but the students would receive benzoic and sorbic acid from other foods permitted to use these additives especially in bakery and snack. Moreover high benzoic acid exposures were found in the beverage that student like to consume including non-carbonated water-based flavoured drinks, and carbonated water-based flavoured drinks, in particularly at the 97.5th percentile exposure (eater only) contributed as 43.3 and 30.4 % of ADI respectively. Therefore the children should not consume large amounts of these beverages.

Keywords: Dietary exposure, Benzoic acid, Sorbic acid, Non-alcoholic beverages

EXPOSURE ASSESSMENT OF BENZOIC ACID AND SORBIC ACID FROM PROCESSED MEAT AND FISH CONSUMPTION OF NAKHON PATHOM STUDENTS

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Benzoic acid and sorbic acid have not permitted for use in processed meat and fish according to the Thai standard for food additives (the notification No.281). However, benzoic acid and sorbic acid were found in various processed meat and fish products vended in Thai market. High consumption of these products may pose health risk in Thai people with a low body weight especially children. This study aimed to estimate the exposures of benzoic acid and sorbic acid from processed meat and fish consumption in Thai students in order to assess the risk on young consumer's health. The exposures were estimated by combining the food consumption data of Nakhon pathom students (432 students) surveyed in 2011 using 24 h dietary recall (three school days) and the concentration data of benzoic acid and sorbic acid in processed meat and fish products that the students liked to consume (190 samples) determined by high performance liquid chromatographic method. The results showed that the mean exposures to benzoic acid and sorbic acid from processed meat and fish products in primary and secondary students were below the ADI. The important source to benzoic acid exposure was meatballs. Sausages contributed the high sorbic acid exposure in the students. In addition, high consumption of pork meatballs (eater only) with contained the highest benzoic acid level (5,408 mg/kg) provided exposure exceeded the ADI in primary students (141% of ADI) and secondary students (185% of ADI). Thus the use of benzoic acid in meatballs should be strictly controlled. Meatball producer should be encouraged to produce according to the good manufacturing practices especially products vending in school.

Keywords : Exposure assessment, Benzoic acid, Sorbic acid, School students

EPA'S BENCHMARK DOSE MODELING EFFORTS: CURRENT CAPABILITIES AND FUTURE DIRECTIONS

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A benchmark dose (BMD) is the modeled estimate of the dose or concentration that produces a predetermined change in response rate of an adverse effect. The BMD method has many advantages over the more traditional No Observed Adverse Effect Level (NOAEL) method. BMD modeling is the preferred approach used by the United States Environmental Protection Agency (EPA) in human health risk assessments, and EPA has developed a free BMD modeling software package (BMDS) (www.epa.gov/ncea/bmds). BMDS version 2.2 contains thirty different models for the analysis of quantal data, continuous measurement data, and nested developmental toxicology data. BMDS version 2.2 also contains models for multiple tumor analysis and concentration-time data. In addition to BMDS, EPA has developed categorical regression software (CatReg) to analyze dose-time-response data where responses are expressed in terms of effect severity. CatReg allows a meta-analysis of data from multiple studies with multiple endpoints for multiple test species. EPA is considering several improvements to BMDS in order to expand its modeling capabilities, including: using the "hybrid" approach for continuous data; extending a lognormal distribution option to all continuous models; and estimating risk at a specified dose. Long-term considerations for BMD tools include: model averaging approaches; nonparametric Bayesian modeling; addition of covariates to models; use of a bootstrapping method for BMDL estimation; development and estimation of a BMD upper bound confidence limit. In the future EPA will continue to improve BMDS to provide dose-response capabilities to research, industrial, and governmental entities to facilitate robust analysis of multiple types of toxicological data and to support regulatory actions intended to protect human health.

The views expressed in this abstract are those of the authors and do not necessarily represent the views or policies of the U.S. Environmental Protection Agency.

INTEGRATION OF TOXICOGENOMICS DATA IN MODE OF ACTION ANALYSES AND CANCER RISK ASSESSMENTS

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Experimental approaches capable of evaluating a wide range of mechanisms of action are expected to significantly impact human health risk assessment (HHRA). The United States Environmental Protection Agency's (EPA) 2005 Guidelines for Carcinogen Risk Assessment encourages incorporation of data from new technologies to better inform HHRA. Toxicogenomic (TG) data (e.g. changes in gene expression profiles) are beginning to be used in HHRA to inform qualitative mode of action (MOA) analyses and support weight-of-evidence evaluations for the characterization of carcinogenicity of environmental chemicals. One of the hallmarks of TG is identification of molecular signatures, based on gene expression patterns, which discriminate direct-acting genotoxicants from indirect-acting genotoxicants. Quantitative methods for TG data analysis include benchmark dose modeling of dose-responses from microarray data. This method analyzes changes in transcriptional profiles following chemical exposure both in individual genes as well as families of genes associated with specific cellular processes then calculates benchmark doses to identify the most susceptible single gene or cellular process. These quantitative methodologies can be informative in the determination of carcinogenic MOAs. This presentation summarizes current scientific progress in TG methodologies and presents two examples (naphthalene and polycyclic aromatic hydrocarbons) demonstrating how TG data could be incorporated into HHRA analyses. The information obtained from such analyses can be utilized to improve understanding of the MOA for environmental carcinogens and to facilitate better cancer risk characterization.

The views expressed are those of the authors and do not necessarily reflect the views or policies of the U.S. EPA.

UTILITY OF THE UNITED STATES ENVIRONMENTAL PROTECTION AGENCY'S INTEGRATED RISK INFORMATION SYSTEM (IRIS) IN HUMAN HEALTH RISK ASSESSMENT

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Human health risk assessment is the technical analysis of the nature and magnitude of the human health risks posed by exposure to environmental contaminants. The mission of the United States Environmental Protection Agency (U.S. EPA) is to protect public health and the environment. Under the authority of a series of environmental laws, the U.S. EPA limits human exposure to environmental contaminants by regulating releases of chemicals and other substances to the air, water, and land, and by reducing concentrations of contaminants already in environmental media. Risk assessment plays a unique role in serving the needs of the U.S. EPA and various international programs by incorporating, integrating and coordinating the use of scientific information to serve as a foundation for regulatory decision-making. The U.S. EPA's Integrated Risk Information System (IRIS) provides a major component of the human health risk assessment process via the development of IRIS assessments. IRIS evaluates toxicity information for chemical contaminants found in air, water, or soil and provides state-of-the-art hazard identification and quantitative dose-response assessments. IRIS assessments can be combined with site- or problem-specific exposure assessments to estimate risks to human health. The IRIS database is located on the internet at www.epa.gov/iris and contains assessments for more than 540 chemical contaminants. The IRIS database is one of U.S. EPA's more highly accessed web sites and provides risk assessment information to the world with users in more than 100 countries accessing the IRIS database on a regular basis.

(The views expressed in this abstract are those of the author and do not necessarily reflect the views or policies of the U.S. Environmental Protection Agency.)

EVIDENCE FOR SAFETY OF CHRONIC USE OF AYURVEDIC HERBOMETALLIC AND BHASMA PREPARATIONS

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Arogyavardhini vati (herbometallic) and Sidh Makardhwaj (bhasma) are mercury based preparations mentioned in Ayurvedic formulary of India used for the treatment of liver and neurological disorders respectively. However, recent reports of high concentration of heavy metals in Ayurvedic or herbal preparations have raised much concern and controversy. Therefore, the present study was designed to evaluate the safety profile of chronic administration of Arogyavardhini vati (AV) and Sidh Makardhwaj (SM) in rats. The equivalent therapeutic dose of AV and SM in rat was calculated as 50 & 10 mg/kg. Graded doses of AV (50, 250, 500 mg/kg) and SM (10, 50, 100 mg/kg), mercury chloride (1 mg/kg) and normal saline were administered orally to male Wistar rats for 28 days. Behavioral parameters were assessed on day 1, 7^{th}, 14^{th} and 28^{th} using Morris water maze, passive avoidance, elevated plus maze and rota rod. Liver and kidney function tests were done on 28^{th} day. Animals were sacrificed and brain acetyl-cholinesterase activity, levels of malondialdehyde (MDA), reduced glutathione (GSH) in brain, liver, kidney were estimated. The levels of mercury in brain, liver, kidney were estimated by ICP-AES. Histopathology of these tissues was also performed.AV and SM in the doses used mentioned above did not cause significant change in neurobehavioral parameters, brain AChE activity, liver (ALT, AST, ALP bilirubin) and kidney (serum urea and creatinine) function tests as compared to control group. The levels of mercury in brain, liver, kidney was found to be raised in dose dependent manner. However, the levels of MDA and GSH in these tissues did not show any significant change as compared to control. Also, there was no histopathological change in cytoarchitecture of brain, liver, kidney. The findings of the present study suggest that Arogyavardhini vati and Sidh Makardhwaj upto 10 times the equivalent dose administered for 28 days did not show any toxicological effects on brain, liver and kidney.

EVALUATING THE SAFETY OF BOTANICAL RAW MATERIALS USED IN COSMETICS

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There is a growing trend of using botanical raw materials in personal care products, such as those used in traditional medicine or those from the many exotic plants which may be part of local folklore or food use. Asian countries are both a significant source of suppliers of these botanical ingredients as well as providing the demand from consumers for cosmetic products containing them. These materials present a challenge to both the *Product Developer* and *Regulator* alike, in assuring the development of safe cosmetic products, from the complex composition of the materials through to the lack of documented data on history of use and safety. There is demand for some form of guidance in this area to pull together various risk assessment concepts. A critical first step in the risk assessment is the characterisation of the botanical raw material, with key measurements being identified. A complete understanding of "what is known?" about the material should then be developed from both literature sources as well as traditional knowledge. It may then be possible to determine whether a history of safe use (HOSU) can be established and if the material can be considered safe at that stage for particular cosmetic applications. If not then further risk assessment approaches (ie comparative approach, threshold of toxicological concern (TTC)) are proposed. Finally, in order to complete the risk assessment there may be a need to fill gaps in the hazard profile and/or potential consumer exposure scenarios, by conducting some further testing.

PHTHALATE REPRODUCTIVE TOXICITY: IMPLICATIONS FOR CUMULATIVE RISK ASSESSMENT

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People are exposed to diverse chemicals from multiple sources in daily life. It has long been understood that chemicals interact in the body and combined exposure to chemicals could affect and change the health effect. However, until now, chemical risk assessment generally has been to consider single chemical separately and typically from a single source. Recently, the problem of combined exposures and health risks has become greater concern for regulatory authorities. To ensure timely consideration, KFDA is taking approaches on the development and improvement of risk assessment procedure for combined exposures to multiple chemicals. As the first step, we are reviewing available methods and tools for assessing the combined risk and developing working definitions for different types of exposures, effects and risks of multiple chemicals. We also focus on developing probabilistic models based on those from other authorities considering Korean dietary and environment. The main goal of this project is to develop a guideline for risk assessment of combined exposure built on the previous guidance, which was published to aid risk assessors in identifying priorities for risk management. In addition, this research annexes a case study of phthalate to test and refine the framework. Phthalates are used extensively in cosmetics, medications, and food packaging. It is clear that human health risk assessment methodologies need to address not only exposures to single phthalates, but also to mixtures of phthalates and other chemicals. Furthermore, to discuss and review available methods for assessing the combined risk via all relevant routes, a committee is convened among KFDA, National Institute of Environmental Research, Animal Plant and Fisheries Quarantine and Inspection Agency and Korea Centers for Disease Control and Prevention. The committee is expected to fill gaps in lacking data, find adequate methodologies and gain knowledge in different sectors including occupational, environmental and consumer exposure.

INHIBITION OF GROWTH AND SPORE GERMINATION OF *ASPERGILLUS FLAVUS* MUCL 18820 BY LACTIC ACID BACTERIA ISOLATED FROM THAI FERMENTED FOOD

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Aflatoxins (AF) are naturally occurring mycotoxins, highly toxic and among the most carcinogenic compounds. They are involved in hepatocarcinoma in Africa and Asia. AF are synthesized by several Aspergilli, mainly by *Aspergillus flavus*. On method to avoid contamination is to hinder fungi development. One hundred and nine lactic acid bacteria were isolated from Thai fermented food. The isolates were tested for growth inhibition of *Aspergillus flavus* MUCL 18820. It found that 31 isolates gave a good inhibition on mould growth during 6 days of incubation using Modified overlay method. Nine isolates, including H4, E6, F5, I3, K2, I8, D2, 4 and 15, out of 31 isolates inhibited mycelium growth of *A. flavus* MUCL18820 on Potato Dextrose Agar. These 9 isolates were then tested for spore germination inhibition in Potato Dextrose Broth. The resulted indicated that only H4 and 15 gave 100% inhibition within 4 days of determination. Higher concentration of LAB resulted in better inhibition of mould growth and spore germination. The 2 isolates were identified as *Lactobacillus plantarum* (%ID = 99.9) according to API 50 CH test.

Keywords*:* Lactic acid bacteria, Aflatoxin, *Aspergillus flavus*

BINDING CAPACITY OF LACTIC ACID BACTERIA OR YEAST- BY- PRODUCT FOR ZEARALENONE DETOXIFICATION

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Zearalenone is a powerful natural phytoœstrogene. This mycotoxin is synthetised by *fusarium*, and is deleterious in pig and poultry industries. It is an endocrine disruptor which could affect also human helath. This research aimed to study the efficacy of lactic acid bacteria (LAB) or yeast-by-products to detoxify zearalenone (ZEA). Nine strains of LAB, namely *Lactobacillus pentosus* DM068, *L. pentosus* JM085, *L. pentosus* JM0812, *L. pentosus* VM095, *L. pentosus* VM096, *L. pentosus* UM054, *L. pentosus* UM055, *L. pentosus* YM122, and *Enterococcus faecalis* YM126, were used. Yeast cell walls (YCW) from baker or brewer industry, alive yeast, yeast enriched in glutathione (GSH) or selenemethionine (SeMet) were tested. ZEA binding capacity of LAB depended on tested strains and ZEA concentration in buffer solution. At the highest concentration of ZEA (75 µg/ml), 4 LAB strains showed binding ability above 80%; as followed, *E. faecalis* YM126 (87.26%), *L. pentosus* JM0812 (83.17%), *L. pentosus* UM054 (82.77%), and *L. pentosus* UM055 (81.69%). At 50 µg/ml ZEA , 3 strains gave binding capacity higher than 70%; for example, *L. pentosus* DM068 (75.17%), *L. pentosus* VM096 (70.38%), and *L. pentosus* UM055 (69.98%), respectively. When the concentration was between 1-20 µg/ml, LAB were able to bind ZEA only 40%. Depending of the yeast by product and whatever the ZEA concentrations the binding ranged between 30-70%. The best adsorption was obtained with baker's YCW and Yeast enriched in GSH or SeMet and the lowest adsorption was observed with brewer'sYCW.

Keywords: Lactic acid bacteria, Zearalenone, Binding

FORGING INTERNATIONAL PARTNERSHIPS FOR ADVANCING HUMAN HEALTH RISK ASSESSMENT

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The mission of the U.S. Environmental Protection Agency (EPA) is to protect human health and the environment. EPA limits human exposure to environmental contaminants by regulating releases of chemicals and other substances to the air, water, and land, and by cleaning up contaminants already in environmental media. Human heath risk assessment provides the scientific basis for EPA's decisions about chemicals in the environment. EPA's Human Health Risk Assessment (HHRA) program provides state-of-the-art hazard identification and dose-response assessments – a critical component of a full risk assessment, and one of the key scientific components underpinning the Agency's decisions about chemicals in the environment. When hazard and dose-response assessments are combined with site- or problem-specific exposure assessments, risk assessors in the developed and developing countries can develop estimates of risk that inform decisions about chemicals in the environment, such as acceptable levels of chemicals in drinking water and clean-up levels at waste sites. Tools and information developed by the HHRA program are used by risk assessors and risk managers at EPA and beyond to make decisions about cleaning up the environment, helping to ensure that decisions are based on the highest quality, peer reviewed scientific information available (http://www.epa.gov/riskassessment/). Currently there are several challenges facing the international risk assessment community in both developing and developed countries. For example, there are tens of thousands of chemicals in commerce and the environment that are untested and lack assessment of their potential to cause human toxicity. Additionally, traditional toxicology testing methods are expensive, slow, and cope with too few chemicals. Finally, toxicology approaches are evolving away from reliance on in vivo testing of laboratory animals and moving toward in vitro and in silico approaches. Current approaches to human health risk assessment must be modified to address these challenges, allowing the risk assessment community to assess more chemicals more quickly using fewer resources. EPA's HHRA program is evolving to address these challenges and in the face of new types of data and new understandings about uncertainty, mode of action, metabolism, susceptibility, and other emerging scientific issues. This presentation will: 1) provide an overview of EPA's Human Health Risk Assessment program; 2) explain the important role it plays in the risk assessment community; 3) highlight tools produced by the program for the risk assessment community; and 4) illustrate how the program is evolving to address current risk assessment challenges, both in the U.S. and internationally. Opportunities for international collaboration will also be explored.

BISPHENOL A AND PHTHALATE INDUCED THE GROWH OF HUMAN PROSTATE CANCER LNCaP CELLS AND ALTERED TGF-BETA SIGNALING PATHWAY VIA ESTROGEN RECEPTOR AND ANDROGEN RECEPTOR-DEPENDENT MANNERS

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Endocrine disrupting chemicals (EDCs) can bind to the hormone receptor and induce the unexpected hormone response to activate estrogen receptor (ER) and androgen receptor (AR) mediated signaling pathways, which have potential to effect on the hormone-dependent carcinogenesis. As prostate cancer progression, steroid hormones, specifically androgen, is important factor in cancer growth and diagnosis. Among EDCs, Bisphenol A (BPA) has the detrimental effect on the endocrine system and is suspected to promote human breast and ovarian cancer. Recent studies have reported that phthalate can disrupt endocrine system and has weak estrogenic activity with binding to ERs. Thus, we demonstrated in this study whether BPA and dibutyl phthalate (DBP) stimulate the proliferation of prostate cancer cells, LNCaP cells having both ERs and ARs. We evaluated proliferation rate of LNCaP cells following BPA and DBP treatment using a cell viability assay compared to EtOH. Both BPA and DBP increased LNCaP cells proliferation over two-fold at 10^{-7} M to 10^{-5} M. Moreover, these EDCs altered translational expression of cell clycle related genes, *cyclin D1* and p21 at 6 h in LNCaP after exposure of BPA and DBP. Overexpression of Cyclin D1 and downexpression of p21 can rapidly transit G1/S phase during the cell cycle. These effects of EDCs reduced in LNCaP cells treated by ER antagonist and AR antagonist. Futher, we examined the alteration of gene expression of *c-myc* and *c-fos* by using the semi-quantitative RT-PCR. Like 17 β-estradiol (E2) and dihydrotestosterone (DHP), treatments of BPA and DBP lead to increase the transcriptional levels of *c-myc* and *c-fos* in LNCaP cells from 30 min to 6 h. In addition, BPA and DBP decrease the protein level of not only *p-smad* but also *total smad*. These facts show effects of EDCs on TGF- β signaling in cancer. Taken together, these results suggest that BPA and phthalate can alter various gene expressions in TGF- β signaling and stimulate cell growth in prostate cancer cells *in vitro*. A futher study warranties to determine the potential of EDCs in the carcinogenesis of prostate cancer *in vivo.*

Keywords: Bisphenol A, pthalate, prostate cancer, estrogen and estrogen receptors

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BAICALEIN ACTIVATES ARYL HYDROCARBON RECEPTOR AND DECREASES THE EXPRESSION OF CDK4 AND CYCLIN D1 TO INDUCE G1 PHASE ARREST IN ORAL CANCER CELLS

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Baicalein is a flavonoid known to have anti-inflammatory and anti-cancer effects. Its function as an aryl hydrocarbon receptor (AhR) ligand that modulates AhR-mediated dioxin toxicity was recently proposed. This study investigated if the antiproliferative effect by baicalein was mediated by AhR. The results from MTT assay showed baicalein at concentrations of 7 to 56 μg/ml inhibited cell proliferation in a dose-dependent way in oral cancer cells, HSC-3. Cell cycle analysis showed that baicalein (28μg/ml) arrested cells at G1 phase. The G1 phase arrest in baicalein-treated cells, through the detection of Western blot method, was associated with decreased CDK4, cyclin D1 and retinoblastoma (Rb) phosphorylation. Results from luciferase assay revealed that baicalein activated AhR activity in HSC-3. Furthermore, the hypophosphorylation of Rb was partially reversed when using siRNA to suppress the expression of AhR in baicalein-treated cells. However, the reduction of CDK4 and cyclin D1 by baicalein was not different between cells with or without AhR knockdown. These data indicate that baicalein inhibits cell proliferation by causing cell cycle arrests at G1 phase. The molecular mechanism of baicalein on G1 phase arrest is mediated by the hypophosphorylation of Rb. The activation of AhR, and the reduction of CDK4 and cyclin D1contributes to the de-phosphorylation of Rb in baicalein treated cells. The data of the study suggest that baicalein can be a chemopreventive agent to inhibit AhR- and cyclin D1-associated cancer cell proliferation.

THE STABILITY OF ANTIOXIDANT ACTIVITY AND ANTIMUTAGENICITY OF RAW OR HEAT-PROCESSED EDIBLE EGGPLANT (*SOLANUM MELONGENA L.*)

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Solanum melongena L. (Ma Khuea Muang Glom) was separated into three groups. The first group was lyophilized and kept as untreated control sample. The second group was steam-blanched for 2 min and lyophilized; half of it was kept as the second sample and the other half was fried at 120-140˚C for 10 min in palm oil and served as the third sample. Three-day old trans-heterozygous larvae (*mwh flr*$^{+}$*/mwh TM3*) were transferred to an experimental medium (containing a sample) that had 20 mM urethane as the positive mutagen. The wings of surviving flies were analyzed for occurrence of mutant spots. The results showed that all treated samples had both antimutagenicity and antioxidant activity (determined using DPPH assay and ferric reducing antioxidant power (FRAP) assay) and phenolic compounds (determined using Folin-Ciocalteu reagent) with different degrees of content. *Solanum melongena* is, in fact, a source of chlorophylls. Therefore, chlorophylls converted into pheophytin, pyropheophytin, and pheophorbide during heat processing were thought to act as antimutagens in this study.

ANTIMUTAGENICITY OF BANANA, MANGO AND PAPAYA RIPENED CONVENTIONALLY OR ARTIFICIALLY (USING ACETYLENE OR ETHYLENE GAS) AGAINST URETHANE INDUCED WING SPOT IN *DROSOPHILA MELANOGASTER*

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Banana (Kluay-Nam-Wa variety), mango (Chok-Anan variety) and papaya (Holland or Plak-Mai-Lai variety) harvested at green mature stage were conventionally ripened or artificially ripened using either ethylene generated by ethephon or acetylene generated by calcium carbide moistened with water. In the antimutagenicity evaluation, the three-day old trans-heterozygous (*mwh flr*$^{+}$/*mwh TM3*) larvae were transferred to an experimental medium (made by substituting each lyophilized sample for corn flour) containing 20 mM urethane (URE). The wings of the surviving flies were analyzed for the occurrence of mutant spots. The antioxidant activities analyzed by DPPH and FRAP assays and total phenolic contents measured by calorimetrically using Folin-Ciocaltau reagent were also done. The mutation index (MI) of urethane expressed as the ratio of number of spots per wing induced with URE in the presence of sample to number of spots per wing induced with URE alone was used to determine the modifying effect of treated sample on urethane mutagenicity. MI equal to 1 means no interaction while the antimutagenicity or potentiating effect is pronounced when MI is less or higher than 1, respectively. Artificial ripening increased the antimutagenicity of mango because the MI of urethane in the presence of each treated mango decreased to 0.92 (conventional), 0.69 (acetylene) or 0.77 (ethylene). Ripened banana and papaya had potentiating effect on urethane mutagenicity. There was no difference in DPPH radical scavenging activity between conventional and artificial ripening treatments but FRAP value and total phenolic contents decreased in artificial ripening, except that of mango. The interaction between high sucrose (determined as total soluble solids of each sample) and P-450 enzyme activation leading to an increase formation of the active metabolite might be the explanation of the results as such.

ANTIOXIDANT ACTIVITY AND EFFECT ON URETHANE INDUCED MUTAGENICITY IN *DROSOPHILA MELANOGASTER* OF SOME THAI DESSERTS AND SNACKS

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Thai desserts namely, santol compote (Kra Thon Loi Kaew), pumpkin cake (Khanom Fak Thong), black sticky rice pudding with taro (Khao Niew Dam Piak Phuek), crispy flower (Dok Jok), mung bean rice crepe (Tua Paep), steamed Thai dessert made of mixture of flours and syrup (Nam Dok Mai), black coconut sweet pudding (Piak Pun), taro paste (Med Kanoon Sai Phuek), deletable imitation fruits (Luk Chup), and crispy gelatin (Wun Krob) and Thai snacks namely, patty shells with minced chicken (Kra Tong Tong), deep fried banana (Kluai Thot), stuffed and steamed Thai pasta (Khao Kreb Pak Mo), crispy rice (Khao Tung Tod), Thai sticky rice steeped in coconut milk (Khao Niew Mun), dumplings with minced pork and chicken (Cho Muang), fried golden bag (Thung Tong) , Thai minced pork and shrimp relish (Ma Ho), leaf wrapped bite size appetizer (Miang Kham), and minced pork and shrimp in a egg net (La Taeng) were determined that they contained phenolic compounds as well as antioxidants determined by DPPH scavenging and FRAP assays. Then, they were evaluated on their antimutagenicity against urethane induced wing spots in *Drosophila melanogaster*. In the evaluation, three-day-old trans-heterozygous larvae (*mwh flr+/mwh TM3*) were transferred to the *Drosophila* medium mixed with each freeze-dried sample (at the ratio of 1:1 w/w) and 20 mM urethane. It was shown that Lukchup had the best antimutagenicity among Thai desserts while Miangkham was named the best Thai snack. The results indicated that Thai desserts and snacks contained antioxidants (determined by DPPH scavenging and FRAP assays) and phenolic compounds that could scavenge the active free radical product of urethane and/or modulated the biotransformation of urethane in the tester organism.

ANTIOXIDANT ACTIVITIES AND MODULATING EFFECT ON THE MUTAGENICITY OF SODIUM NITRITE TREATED 1-AMINOPYRENE IN AMES TEST OF BANANA LEATHER STORED FOR DIFFERENT PERIODS OF TIME

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The color of fruit leather varies depends on the Maillard reaction products (MRPs) which have been reported in many studies to be high antioxidant capacity and a bifunctional property of comutagenicity and antimutagenicity. In the present study, banana was firstly made to be puree containing added sucrose. Then it was heated with stirring at 90°C for 15 min and was spread in a hot-air oven at 75°C until the water activity was less than 0.6 to be banana leather. The banana leather was stored at 25°C for a certain period of time (0, 45, 90 and 135 days). The color of banana leather was darker than its corresponding freeze dried one; it is possibly due to the formation of MRPs. An increase in darkness of banana leather was observed during storage while the total phenolic contents of banana leather and the antioxidant activity determined by FRAP assay decreased while the antioxidant activity determined by DPPH scavenging assay was not changed as compared to its corresponding freeze dried one. It was found that banana leather and freeze dried banana were antimutagenic against the products of 1-aminopyrene treated with sodium nitrite through both *Salmonella typhimurium* strains TA98 (frame-shift mutation) and TA100 (base-pair substitution mutation) of the Ames test but at the lesser extent than that of the freeze dried one. The possible mechanism of antimutagenicity of the samples may relate to phenolic compounds which some of them e.g. flavonoids might inhibit bacterial enzymes required for the activation of the standard mutagen.

ANTIMUTAGENIC EFFECTS OF *ANNONA SENEGALENSIS* FRUIT EXTRACTS

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Mutagenesis is one of the major contributing factors to carcinogenesis. Although many chemicals can also contribute to the carcinogenic process without inducing mutations, it yet remains a fact that many cancers are initiated by DNA alterations in the target cell that arise as a result of a lifetime's exposure to various environmental factors, in combination with genetic factors. The search for inhibitors of mutagenesis may therefore be useful as a tool to discover chemopreventive agents that could be used to reduce the risk arising from exposure to various environmental mutagens by affecting various stages of cancer development. Such inhibitors have already been isolated from natural sources including plants. Methanolic extracts from the fruit of *Annona senegalensis* Pers. (wild custard apple) were investigated, using Ames test, for their antimutagenic effects as part of study exploring the possibility of using edible fruits as antimutagenic agents. The Ames test was performed with *Salmonella typhimurium* strain TA98 and TA100. Extracts from the fruit flesh, fruit skin and seeds of *A. senegalensis* had moderate antimutagenic activity against the environmental mutagen 4 NQO when TA100 was used (37.5%, 36% and 39.5% respectively), while only extracts from the flesh and the seed had antimutagenic effects against TA98 (41 and 44% respectively). Confirmation of the results in the micronucleus/cytome and comet assays is in progress.

ANTIHYPERTENSIVE ACTIVITY OF *HIBISCUS SABDARIFFA* AQUEOUS CALYX EXTRACT IN *ALBINO* RATS

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The anti-hypertensive activity of aqueous calyx extract of *Hibiscus sabdariffa* on salt induce hypertensive albino rats was investigated for 28 days. The extract and drug treated groups showed a significant ($P<0.01$) reduction in diastolic & systolic blood pressured when compared to the normotensive and hypertensive rats. There was no significant difference ($P<0.05$) between the drug treated group and the extract treated groups during this treatment. Thus this study provided a scientific basis for the use of *H. sabdariffa* calyx extract in the treatment of hypertension.

Keywords: anti-hypertension, *H. sabdariffa* calyx, diastolic, systolic and blood pressure

COLCHICINE PROTECTS DOPAMINERGIC NEURONS IN A ROTENONE MODEL OF PD IN RATS

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Parkinson's disease (PD) is a neurodegenerative disease characterized by dopaminergic (DA) neuronal cell loss. Besides inflammation, oxidative stress and apoptosis, a recent hypothesis suggested that degeneration in DA neurons occurs secondary to abnormal mitosis in these 'postmitotic neurons' ending up with apoptosis. As such, the use of antimitotic drug would be a possible therapeutic approach. Besides its anti mitotic effects, colchicine has anti-inflammatory, anti-oxidant and anti-apoptotic effects. Moreover, clinical surveys proved that patients receiving colchicine for treating musculoskeletal disorders have lower incidence of PD. In addition, the difficult penetration of colchicines to the blood brain barrier disappears in PD patients due to depression of the p-glycoprotein efflux system in these patients. Based on these data we explored the neuroprotective effects of colchicine on rotenone induced PD model in rats. Methods: 30 *Sprague Dawley* rats aged 3 months were divided into 3 equal groups. The first received daily intraperitoneal injections of 0.5% carboxymethyl cellulose (CMC) 3mL/Kg. The second group received rotenone suspended in 0.5% CMC intraperitoneally at a dose of 3 mg/kg, daily. The third group received the same rotenone regimen plus daily oral colchicine at a dose of 20µg/kg. All animals were evaluated regarding locomotor disturbance through blinded investigator who monitored akinesia, tremors and performance on grid test. After 35 and 70 days the animals were sacrificed and their brains were immunostained against anti-TH antibodies. Photomicrographs for coronal sections of SNpc and striatume were taken and analyzed using image J software to evaluate cell count in SNpc and striatal fibers density. The results were then analyzed statistically. Results: showed protective effects of colchicine against rotenone induced neurotoxicity as evident by behavioral tests and immunostaining analysis. Conclusion: Colchicine can offer neuroprotection against neurotoxic effects of rotenone on DA neurons.This suggests possible clinical application of colchicines in treating PD patients.

ANTI-OXIDANT AND ANTI-INFLAMMATORY ACTIVITIES OF *PSEUDERANTHEMUM PALATIFERUM* (NEES) RADLK. ETHANOL EXTRACT

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Hoan-Ngoc (*Pseuderanthemum palatiferum*), a well-known Vietnamese traditional medicine in Thailand, has been shown to exhibit anti-oxidant, anti-bacterial and anti-diabetic effects. Hoan-Ngoc leaves have long been used in folk medicine for curing inflammation associated with wound healing, general trauma, colitis and nephritis. The present study aimed to investigate the anti-oxidant and anti-inflammatory properties of the ethanolic extract of Hoan-Ngoc leaves (EE) in murine macrophages RAW264.7. The results suggested that EE decreased reactive oxygen species generation in 2′,7′-dichlorofluorescien diacetate–loaded RAW264.7 cells. As regards nitric oxide (NO) production, EE demonstrated a dose-dependent suppression of NO production in LPS plus IFN-γ stimulated RAW264.7 cells. In addition, EE also attenuated the productions of LPS-induced tumor necrosis factor (TNF)-α and Interleukin-6 (IL-6). These results suggest that EE may provide potential protective effects against free radicals and modulate excessive inflammatory molecules (NO, TNF-α and IL-6) in response to inflammatory stimuli. Therefore, Hoan-Ngoc leaves may be a promising candidate for future anti-inflammatory drug development.

IMPROVEMENT OF FERMENTED THAI RICE NOODLE (KHANOMJEEN) WITH NATURAL COLORS AS A SOURCE OF ANTIOXIDANT

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The water extracts of ten natural color sources, namely *Aegle marmelos* Corr. (bael fruit), *Oryza sativa* L. indica (black rice), *Clitoria ternatea* Linn. (butterfly pea flower), *Daucus carota* Linn. (carrot), *Chrysanthemum indicum* (chrysanthemum flower), *Curcuma longa* Linn. (turmeric), *Hylocereus polyrhizus* (dragon Fruit), *Pandanus odorus* Ridl. (fragrant screw pine), *Hibiscus sabdariffa* Linn. (roselle flower) and *Carthamus tinctorius* Linn. (safflower flower) were incorporated into Khanomjeen (Thai rice noodle). The antioxidant activity of methanolic extract from each sample was investigated by using scavenging capacity (DPPH) and Ferric Reducing Antioxidant Power (FRAP). Most colored Khanomjeen had higher antioxidant activity and amount of phenolic compounds than that of the original Khanomjeen. Black rice Khanomjeen revealed its highest antioxidant activity while carrot Khanomjeen showed the lowest activity. The extract of colored Khanomjeen that exhibited high antioxidant activity had relatively high total phenolic content. The correlation coefficient (*r*) between antioxidant activity expressed as TEAC and total phenolic content of Khanomjeen extracts is 0.7698, and between antioxidant activity expressed as FRAP value and total phenolic content is 0.7734. The highest phenolic content and highest antioxidant activity were found in black rice Khanomjeen, roselle flower Khanomjeen and chrysanthemum flower Khanomjeen, respectively. It is, thus, clear that colored Khanomjeen are potential sources of natural antioxidants.

Keywords: Natural color, Phenolic, Antioxidant, Fermented Thai rice noodle

ANTIMUTAGENIC AND CO-MUTAGENIC ACTIVITIES OF SOME LEGUME SEEDS AND THEIR SEED COATS

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This study aimed to determine the phenolic content and antimutagenicity of some legume seeds and seed coats (black bean, mung bean, peanut, red kidney bean and soybean). The raw red kidney bean exhibited the highest phenolic content (103.2 ± 6.2 mg gallic acid equivalents (GAE)/g extract). The seed coats of black bean, peanut and red kidney bean showed high levels of phenolic content (> 200 mg GAE/g extract). This study also determined the antimutagenicity of legume seeds and seed coats against urethane induced somatic mutation and recombination in *Drosophila melanogaster*. As a result, legume seeds of red kidney bean showed the highest antimutagenicity (57.2%), followed by peanut (54.0%). Seed coats extracts at the lowest concentration exhibited weak antimutagenic activity (6.2- 38.8%). The presence of phenolics in the extracts may be responsible for antimutagenicity against urethane. They may induce phase II detoxification enzymes such as glutathione transferase. Moreover, they may also inhibit specific cytochrome P450s, which in turn leads to protection against mutagenesis by decreasing the metabolic activation of urethane. However, at the highest concentration, all seed coats extracts exhibited a synergistic effect on the mutagenicity of urethane. The finding from this study suggested that the antimutagenic/co-mutagenic activity depends upon the levels of phenolics.

Keywords: Legumes, Phenolics, Antimutagenicity, Co-mutagenicity, SMART

PROTECTIVE EFFECTS OF FRUIT EXTRACTS AGAINST FERRIC-NITRILOTRIACETATE INDUCED OXIDATIVE STRESS IN HUMAN HEPATOCYTES

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Iron overload in the body can be highly toxic. The toxicities of excess iron involve many vital organs including liver. Cell or tissue damages associated with iron overload result primarily from free radical reactions and oxidative injury mediated by iron. In this study, six Thai fruits selected based on their chemical antioxidant activities; strawberry, star fruit, guava, longkong, pomelo and tangerine were examined for the ability to improve oxidative damage and enhance cell viability in human hepatocytes, HepG2 cells, which were overloaded with iron from ferric-nitrilotriacetate (Fe-NTA) complex. Fe-NTA enables hepatocytes to accumulate substantially redox-active iron and stimulates the production of injurious hydroxyl radicals, which in turn, initiate oxidative stress-mediated cytotoxicity. Therefore, the effects of fruit extracts on cell survival, ROS generation, lipid peroxidation, and induction of antioxidant enzyme in HepG2 cells subjected to Fe-NTA induced oxidative stress were evaluated. The results showed that the non-toxic concentrations of fruit extracts protected HepG2 cells against oxidative damage induced from Fe-NTA by decreasing intracellular ROS generation and significantly inhibiting lipid peroxidation production. No significant difference increases in the SOD induction were observed in cells co-incubated with Fe-NTA and fruit extracts compared to cells treated with Fe-NTA alone. The study suggests that the extracts of all selected fruits may be useful in protecting against Fe-NTA induced oxidative liver cell damage.

Keywords: Antioxidant, Cytoprotective,Oxidative stress, HepG2 cells, Fe-NTA, Thai fruit

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PHARMACOKINETICS OF OPIATE ALKALOIDS IN EXPERIMENTAL ANIMALS' BLOOD AND BRAIN REGIONS

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The aim of this study was to examine the pharmacokinetics of opiate alkaloids in experimental animals' blood and brain regions (known for their high density of μ-opiate receptors): cortex, brainstem, amygdala and basal ganglia in different time periods (5, 15, 45 and 120 min) after their treatment with heroin. Experiments were carried out on Wistar rats of both genders, which were treated with heroin seized by police authorities at the illegal heroin market in Novi Sad, Serbia. Opiate alkaloids: codeine, morphine, acetylcodeine, 6-acetylmorphine and 3,6-diacetylmorphine were quantitatively determined in blood and brain regions by gas chromatography–mass spectrometry (GC–MS) method. Maximal contents of opiate alkaloids in blood of animals of both genders were found 15 min after the treatment. The values measured in males (20.6±12.5 - 1375.3±220.8 ng/ml) being significantly higher than in females (18.4±1.6 - 748.3±97.9 ng/ml), which suggests a faster passage of opiate alkaloids from blood to brain in female animals. The highest content of opiate alkaloids in brain regions of females was measured 15 min (10.8±6.6 - 484.9±86.8 ng/g) and with males 45 min (4.4±1.4 - 581.3±93.5 ng/g) after the treatment, which also indicates faster distribution of opiate alkaloids from blood to brain in the female compared to male rats. Maximal content of opiate alkaloids was determined in the basal ganglia of both males (20.8±8.2 - 516.3±76.6 ng/g) and females (16.6±5.1 - 484.9±86.8 ng/g), which indicates that this brain region is a reliable sample for identifying and assessing contents of opiate alkaloids after heroin intake. Our results are in agreement with the studies reporting gender differences in a number of the aspects of the pharmacology of opiates. If above mentioned gender differences prove to be valid in humans, the findings could have great medical importance and should be used in pharmacological and behavioral treatments for addiction.

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SAFETY PHARMACOLOGY STUDY OF XANTHINE DRUGS ON PULMONARY AND CENTRAL NERVOUS SYSTEMS

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Safety pharmacology study is very important tool by way of reducing drug's potential risk. Vital organs or systems, the functions of which are acutely critical for life, such as the cardiovascular, respiratory and central nervous systems, are considered to be the most important ones to assess in safety pharmacology studies. Safety pharmacology study of cardiovascular system is generally known throughout the ICH guideline S7B. But, a couple more systems are in poor information about the method of examination.The CNS stimulators, Xanthine analogs are known for drinks such as caffee, tea and cocoa. Xanthine drugs influence on not only CNS but also respiratory system. Caffeine has the properties of CNS stimulator. In contrast, increase in respiration cleary appears when treated with theophylline.We tested respiratory and CNS safety pharmacological studies on 5 xanthine analogs(theophylline, caffeine, diprophylline, pentoxifylline, oxtriphylline). No changes of respiratory function parameters were observed in the clinical dose of these chemicals except for theophylline, a positive control. Respiration rate and minute volume were significantly increased in the maximum dosage groups of the two chemicals(caffeine of 6 mg/kg, oxtriphylline of 50 mg/kg). Caffeine(20 mg/kg), the positive control of CNS safety pharmacology tests, increases the body temperature and activities. Other drugs may cause mild degree of CNS is excited. Now to conclude, it is unlikely that xanthine analogs affects the respiratory fuction at the therapeutic dosage levels in human application. But overdose of caffeine and oxtriphylline, over ten times of the therapeutic dosage, may cause tachypnea. So, we recommended adding matters that require attention about respiratory adverse effects in insert papers of these drugs. And further studies were needed about safety profiles including clinical studies.

CLASTOGENIC AND ANTICLASTOGENIC POTENTIAL OF NEEM FLOWERS EXTRACT USING RAT LIVER MICRONUCLEUS ASSAY

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Clastogenic and anticlastogenic effects of neem flowers (*Azadirachta indica* A. Juss) were determined by using rat liver micronucleus assay. Methanol extract of neem flowers (MENF) in 15% Tween 80 at 100 and 500 mg/kg BW were orally given to male Spraque Dawley rats daily for 2 weeks prior to and during repeated administration of diethylnitrosamine (DEN) for 28 days. All rats were sacrificed after the last treatment and hepatocytes were isolated from the liver by collagenase solution without performing liver perfusion, then stained with 4',6-diamidino-2-phenylindole dihydrochloride (DAPI). The incidence of micronucleated hepatocytes was evaluated by fluorescence microscopy. The results showed that MENF had no clastogenic effect in the rat hepatocytes. On the other hand, MENF at both low and high doses reduced micronucleus formation in the rat liver but significantly different was found only in the high dose group. We concluded that MENF at 100 and 500 mg/kg BW had no clastogenicity but it possesses anticlastogenic potential in the rat liver, particularly the high dose.

Keywords: Azadirachta indica, Anticlastogenic, Clastogenic, Liver micronucleus, Rat

EFFECT OF GINKGO BILOBA EXTRACT ON EXPERIMENTAL MODEL OF EPILEPSY IN CHINCHILLAS

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In the last ten years, the ingredients of ginkgo biloba extracts, which have been found by using biochemical analysis, take place significantly in classic approach in medicine. Mostly, active substanties of ginkgo biloba extracts, release its effects on muscarine receptors and less on adrenergic ones. Recently, potential effects of ginkgo biloba on NMDA receptors have been considered and also, it's effects on epileptogenic seizures. The main goal of this research work was to consider effects of ginkgo biloba extracts on experimental model of epilepsy. The research work was maintained on rabbits-type chinchilla. We used official GINGIUM product, which content 40 mg in 1 ml of dry extract of ginkgo biloba leafages. We formed epileptogenic area with stimulation of hippocampus. Bioelectrical activity has been detected 60 minutes before we formed epileptogenic area as well as 90 minutes after it. Ginkgo biloba extract has been given via I.M. injection in single daily dose of 1ml/kg/BW. According to results that we found in our research work, we could conclude that ginkgo biloba extracts posses proconvulsive activity.

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PROTECTIVE EFFECT OF POMEGRANATE SEED OIL AGAINST CCl_4-INDUCED HEPATOTOXICITY IN RATS

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Pomegranate (*Punica granatum* Linn, *punicaceae*) is cultivated in Iran, India, and some Mediterranean countries. Almost all parts of this plant including flowers, bark, root, leaf and fruit juice have therapeutic effects. Pomegranate seed oil (PSO) contains high amounts of conjugated linolenic acids and is believed to have anti-cancer and antioxidant effects. In this study, PSO was evaluated for hepatoprotective effects against CCl_4-induced hepatotoxicity in rats as pretreatment and post treatment. In pretreatment program, PSO was administrated orally at 1 or 2 ml/kg for 3 consecutive days, and on the third day animals were treated with CCl_4 (1.5 ml/kg, i.p.). For post-treatment study, CCl_4 was administrated and animals were treated with PSO orally at 1 or 2 ml/kg for 2 days thereafter. CCl_4 administration decreased the GSH content of liver from 30±2 μg/g to about 12±1μg/g, and increased lipid peroxidation about 7 folds. It also increased liver marker enzymes SGOT and SGPT in serum dramatically, and caused severe histopathological damage such as necrosis, ballooning degeneration and inflammation to score 9. Pretreatment with PSO at 2 ml/kg restored GSH to the normal level and completely prevented lipid peroxidation, and elevation of serum SGOT and SGPT. Histopathological damages were also completely prevented by 2 ml/kg PSO pretreatment (score 1). Pretreatment with 1 ml/kg or post-treatment with 2 ml/kg PSO were also slightly effective against CCl_4, however, post-treatment with 1 ml/kg PSO had no effect on CCl_4 toxicity. Protective effect of PSO is possibly by the content of antioxidants of PSO, or it may prevent activation of CCl_4 by liver enzymes, as post-treatment had no significant effect on CCl_4 hepatotoxicity. The results suggest that administration of PSO could be effective in preventing oxidative stress-induced hepatotoxicity.

HEPATOPROTECTIVE ACTIVITY OF *CAPPARIS SPINOSA* FRUIT EXTRACT AGAINST CCl_4-INDUCED TOXICITY IN RATS

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Capparis spinosa (CS), a member of *Capparidaceae*, is one of the most usual fragrant plants grown in dried regions of west or central Asia including Iran, and Mediterranean area. From centuries ago, CS aeral parts have been used in traditional medicine for different means, and it is currently used in polyherbal formulations for treatment of liver disorders. CS contains some antioxidants such as flavonoids and other polyphenols which play a great role in many pharmacological activities. In this study, the hepatoprotective activity of CS fruit ethanolic extract (CSE) against CCl_4-induced hepatic damage in rats was evaluated. Administration 1.5 ml/kg CCl_4 (i.p.) to rats decreased the GSH content of liver from 30.1±0.9 mcg/g to about 11.7±0.7 mcg/g, and increased TBARS from 0.7±0.1 to 4.4±0.75 ng/mg tissue. It also increased serum liver marker enzymes ALT, AST and LDH, 62, 26, and 5.5 folds, respectively. Severe histopathological damage including fatty changes, necrosis ballooning degeneration and broad lymphocytes and kupffer cells infiltration were evident. Rats received CSE as pre- and post-treatment with 100 or 200 mg/kg by oral rout. Post-treatment with 200 mg/kg CSE for 2 days after administration of CCl_4 completely prevented histopathological damages and returned serum ALT, AST, and LDH to about normal values. It also restored GSH and TBARS levels to 26.5±0.1 mcg/ml and 0.9±0.1 ng/ml, respectively. Post-treatment with 100 mg/kg CSE or pretreatment with 200 mg/kg CSE also significantly prevented CCl_4-induced damages to the liver but not as effective as 200 mg/kg post-treatment, but pre-treatment with 100 mg/kg CSE did not show any significant protective effect. Therefore, administration of CSE extract may be effective for treatment of oxidative liver damages. Lack of effect of CSE as pretreatment may be due to rapid metabolism of antioxidant content of CSE.

HEPATOPROTECTIVE EFFECT OF *PIMPINELLA ANISUM* SEED ESSENTIAL OIL AND EXTRACTS AGAINST CCL4 INDUCED TOXICITY ON HEPG2 CELLS

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Pimpinella anisum (anise) has been used as a traditional medicinal plant, and its seed has been used for treatment of gastric and liver diseases in Iran. The effect of hydroalcoholic and n-hexane extracts and essential oil of the anise seed were investigated on CCl4-induced cytotoxicity on HepG2 cells. Concentrations (1-1000 µg/ml) of essential oil and extracts were added to the cells, 1 h before the addition of 100 mM of CCl4. After 24 hours, the cells were evaluated for cell toxicity, GSH content and TBARs level. The n-hexane extract with concentrations of 10-50 µg/ml protected the cells against CCl4-induced cytotoxicity, but the hydroalcoholic extract and essential oil with the same concentrations did not have significant protective effect. The anise extracts and essential oil had toxic effect towards cells with concentrations up to 100 µg/ml. Therefore, the results of the present study are somehow in consistent with traditional beliefs about antitoxic effect of Pimpinella anisum.

DIETARY INCLUSION OF ISOFLAVONES-ENRICHED SOY PROTEIN INHIBITS LIPID PROXIDATION AND OXIDATIVE STRESS INDUCED BY AFLATOXIN

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Aflatoxins (*Aspergillus flavus* toxins) are one of the natural toxins produced by a group of *Aspergillus*. These toxins are leading cause of some liver cancers and serious gastrointestinal problems in developing countries. They are well known mutagenic, carcinogenic, hepatotoxic and immunosuppressive. The aim of the current study was to evaluate the protective effects of isoflavones-enriched soy protein against aflatoxin-induced oxidative stress in rats. Four experimental groups treated for 8 weeks included: the control group, soy-supplemented diet (20% w/w) group, the group fed aflatoxin-contaminated diet (2mg AFs/kg diet) and the group fed soy and aflatoxin-contaminated diet. Blood and liver tissue samples were collected for biochemical analyses and histological examination. The results indicated that soy contains 45.8% protein and 167.3 mg/100 g soy total isoflavones. Animals fed AFs-contaminated diet showed a significant biochemical changes in serum accompanied with severe oxidative stress and histological changes in liver. Supplementation with soy succeeded to restore the elevation of liver enzymes activities and improved the serum biochemical parameters. Moreover, soy supplementation improved the antioxidant enzymes, decreased lipid peroxidation and improved the histological picture of the liver tissue. It could be concluded that soy protein enriched isoflavones may be a promising agent against liver diseases.

Keywords: aflatoxins, oxidative stress, soy, liver

GENISTEIN, A NOVEL PHYTOESTROGEN, EFFECTIVELY SUPPRESSED THE GROWTH OF ESTROGEN-DEPENDANT BG-1 OVARIAN CANCER CELLS INDUCED BY 17BETA-ESTRADIOL OR BISPHENOL A, AN ENDOCRINE DISRUPTING CHEMICAL

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Bisphenol A (BPA) is a widely used industrial compound, and also known as one of endocrine disrupting chemicals (EDCs) and especially a xenoestrogen, which is a chemical compound that imitates estrogen in living organisms. 17 β -estradiol (E2), one of estrogens in the body, is a pleiotropic hormone that regulates the growth and differentiation of many tissues and also acts as a mitogen that promotes the development and proliferation of hormone-responsive cancers. In this study, we examined the effect of a phytoestrogen, genistein, on the cell growth of BG-1 ovarian cancer cells induced by the treatment of E2 or BPA. In the cell proliferation test *in vitro*, E2 or BPA increased the growth of the BG-1 ovarian cancer cells expressing estrogen receptors (ERs). Their proliferation activity was reversed by the treatment of ICI 182,780, a well-known antagonist of ERs, which demonstrates that the cell proliferation by E2 or BPA is mediated by ERs and BPA certainly acts as a xenoestrogen in the BG-1 ovarian cancer cells. Genistein, an isoflavone, is one of phytoestrogens that are plant-derived, naturally occuring, and dietary xenoestrogens and influences multiple biochemical functions. In this study, genistein effectively suppressed the BG-1 cell proliferation induced by E2 or BPA by adversely downregulating the cell cycle progression that was upregulated by E2 or BPA. Concretely, E2 or BPA decreased the gene expression of p21, which is a potent cyclin-dependent kinase (Cdk) inhibitor and responsible for the cell cycle arrest at G_1 phase, to proliferate the BG-1 cells. On the other hand, genistein upregulated the expression of p21 gene cultured in the presence of E2 or BPA, leading to the growth inhibition of the BG-1 cells. Also, the alteration of p21 gene expression by E2, BPA, or genistein affected the expression of its downstream genes of cell cycle, cyclin D1 and Cdk-4. Taken together from these results, we may suggest an anticancer effect of genistein, a dietary phytoestrogen, on the estrogen-dependant cancers like ovarian cancer prompted by E2 or BPA.

Keywords: Endocrine disrupting chemicals (EDCs), estrogen (E2), genistein, bisphenol A (BPA), ovarian cancer, p21

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A GROWTH OF BG-1 OVARIAN CANCER CELLS CAUSED BY 17BETA-ESTRADIOL OR VARIOUS ENDOCRINE DISRUPTING CHEMICALS WAS INHIBITED BY RESVERATROL VIA DOWN-REGULATING THE CELL CYCLE PROGRESSION *IN VITRO*

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Endocrine disrupting chemicals (EDCs) appear to promote the development and progression of the estrogen dependant cancers. Resveratrol (trans-3,4',5-trihydroxystilbene, RES), a phytoestrogen, exists in grape skin and red wine. In this study, we evaluated the inhibitory effect of RES on the cell growth and progression induced by various EDCs in BG-1 ovarian cancer cells expressing estrogen receptors (ERs). The various EDCs, i.e., bisphenol A (BPA), nonylphenol (NP), octylphenol (OP), methoxychlor (MXC), and hexabromocyclododecane (HBCD) were employed in this study. In the *in vitro* experiments, treatments of BG-1 cells with E2, BPA, NP, OP, MXC, or HBCD resulted in an increase of their growth. The treatment of BG-1 cells with ICI 182,780, a well known antagonist of ERs, reversed EDCs induced cell growth in these cells, indicating that their growth stimulatory effect is mediated through ERs. In addition, we evaluated the effect of RES in the presence of other EDCs by MTT assay. As a result, increased cell viability induced by these EDCs. On the other hand, cell viability of co-cultured with RES was decreased. In addition, we further examined the regulation of cell cycle dependent genes by RT-PCR in E2, BPA, or NP and mixture of RES and each EDC. Concretely, the treatment with each EDC only decreased the gene expression of *p21* and increased the expression of *cell cycle-dependent kinase 2* (*CDK2)*. However, co-treatment with RES and one of EDCs resulted in the increased gene expressions of *p21* and the decreased expression of *CDK2*. *Cyclin D1* was increased by downregulating *p21* when only treated with each EDC in the absence of RES, while co-treatment with RES and each EDC decreased the gene expression of c*yclin D1* by upregulating *p21*. Taken together, these results indicate that RES appears to be *Cyclin D1* and *CDK2* inhibitor and is responsible for the cell cycle arrest at G_1 phase. In addition, when co-treated with each EDC, RES increased the expressions of *p21* and resulted in the growth inhibition of BG-1 ovarian cancer cells. As a result, we confirmed the cell growth inhibitory effect of RES, a dietary phytoestrogen, on the estrogen-dependent ovarian cancer cells prompted by EDCs.

Keywords: Endocrine disrupting chemicals, resveratrol, estrogen (E2), cell-cycle related genes

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ASSESSMENT OF BIOMARKERS OF BENZENE EXPOSURE AND EFFECT IN PETROCHEMICAL WORKERS

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Background: Benzene is a natural component of crude oil and an essential material for many petrochemical products. The process of benzene production in an aromatic plant facility is in a closed system, and hence occupationally exposure to benzene is low. However, there may be potential benzene exposure during major shutdown due to the release of the chemical especially during maintenance. **Objective**: This study was conducted to assess the biomarkers of exposure and effect, and the relationship between these parameters in the aromatic plant workers during a major turnaround. **Human Subjects**: Pre and post shift blood and urine samples were obtained from 35 workers who were potentially exposed to low air level of benzene including smokers and non-smokers. **Methods**: DNA damage in peripheral blood lymphocytes as the biomarker of effect was analysed by Alkaline Comet Assay. S-phenylmercapturic acid (SPMA) and 8-hydroxyl-2-deoxyguanine (8-OHdG) as biomarker of exposure and effect respectively in urine were assessed by ELISA for pre and post shift samples. Data analysis were performed using the statistical package SPSS (Version 15.0).
Results: The post shift urinary SPMA in the workers demonstrated a 2.1 fold increase compared to the pre shift data. 18 out of 35 workers had levels of post-shift SPMA higher as compared to the pre shift data. Specifically, 6 workers showed levels of SPMA above the Biological Exposure Index (25µg/g creatinine). The strand breaks (Comet assay) and oxidized base (8-OHdG) demonstrated a 2.1 fold and 1.1 fold increase respectively in smokers. In contrast, the non-smokers demonstrated a statistically significant increase in 8-OHdG level (1.3 fold) but not for the DNA strand breaks (1.2 fold). The non-smokers showed a strong correlation between SPMA and 8-OHdG (r = 0.709) in the post shift data.**Conclusion**: Taken together, our findings showed 6 workers had SPMA level exceeding Biological Exposure Index confirming exposure to benzene during the turnaround. However, from this study, the DNA damage (Comet assay) did not correlate with the SPMA level although the 8-OHdG and SPMA level in non-smoker group demonstrated a good correlation in the exposed petrochemical workers.

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CHEMICAL CONSTITUENTS AND ANTIOXIDANT ACTIVITIES OF *CLEISTOCALYX NERVOSUM* FRUITS IN *IN VITRO* AND *IN VIVO* MODELS

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ABSTRACT

Cleistocalyx nervosum var. *paniala*, or Ma-kiang in Thai, is a purplish red fruit cultivated in northern Thailand. Its flesh is edible and is used to make juice, wine and jam. The previous study reported that the aqueous extract of *C. nervosum* pulp showed antioxidant activity and anticarcinogenesis. The present study investigated the fruit's chemical constituents and antioxidant activities, both *in vitro* and *in vivo*. Both aqueous and ethanol extracts (95% v/v) were used. The amount of polyphenols, flavonoids, cyanidin-3-glucoside and DPPH radical-scavenging capacity of the ethanolic extract were greater than those of the aqueous extract. The 95% ethanol extract of *C. nervosum* was further investigated for its safety and antioxidant activity in animal models. The administration of the ethanolic extract of *C. nervosum* at 5 g/kg bw was safe in wistar rats. Various concentrations of *C. nervosum* ethanolic extract at 100, 300 and 1000 mg/kg bw were intragavage fed to male wistar rats for 90 days. The ethanolic extract significantly decreased malondialdehyde (the end-product of lipid peroxidation) levels in liver, but not in serum. It also induced hepatic glutathione peroxidase activity. The results of this study showed that 95% ethanolic extract of *C. nervosum* contains a high content of anthocyanin that has antioxidant capacities both in *in vitro* and *in vivo* models.

Keywords: *Cleistocalyx nervosum,* Anthocyanin, Antioxidant activity

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องค์ประกอบทางเคมีและฤทธิ์ต้านอนุมูลอิสระในหลอดทดลองและสัตว์ทดลองของผลมะเกี๋ยง

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บทคัดย่อ

มะเกี๋ยง (*Cleistocalyx nervosum* var. *paniala*) เป็นผลไม้ที่มีสีม่วงแดงปลูกมากในเขตภาคเหนือของประเทศไทย เนื้อของผลมะเกี๋ยงสามารถรับประทานทั้งในรูปสดและแปรรูปเป็นน้ำผลไม้ ไวน์ และแยม มีรายงานพบว่าเนื้อของผลมะเกี๋ยงที่สกัดด้วยน้ำมีฤทธิ์ต้านอนุมูลอิสระและฤทธิ์ป้องกันการเกิดมะเร็งตับ งานวิจัยครั้งนี้เป็นการศึกษาองค์ประกอบทางเคมีที่สำคัญและฤทธิ์ต้านอนุมูลอิสระของสารสกัดจากผลมะเกี๋ยงทั้งในหลอดทดลองและสัตว์ทดลอง โดยใช้สารสกัดมะเกี๋ยงทั้งที่ได้จากการสกัดด้วยน้ำและ95% เอธานอล จากการศึกษาพบว่าปริมาณสารประกอบฟีนอลิก ฟลาโวนอยด์ ไซยานิดิน-3-กลูโคไซด์(แอนโธซัยยานิน) และฤทธิ์ต้านอนุมูลอิสระในหลอดทดลองโดยใช้วิธี DPPH ของสารสกัดมะเกี๋ยงที่สกัดด้วยเอธานอลสูงกว่าสารสกัดที่สกัดด้วยน้ำ ดังนั้นจึงศึกษาความปลอดภัยและฤทธิ์ต้านอนุมูลอิสระของสารสกัดมะเกี๋ยงที่สกัดด้วยเอธานอลในสัตว์ทดลองต่อไป จากการศึกษาความเป็นพิษเฉียบพลันของสารสกัดมะเกี๋ยงที่สกัดด้วยเอธานอลในหนูวิสต้าร์เพศเมีย 14 วัน พบว่าสารสกัดมะเกี๋ยงความเข้มข้น 5 กรัมต่อกิโลกรัมน้ำหนักตัวมีความปลอดภัยในสัตว์ทดลอง และเมื่อทำการป้อนสารสกัดมะเกี๋ยง 100, 300 และ 1000 มิลลิกรัมต่อกิโลกรัมน้ำหนักตัวให้แก่หนูวิสต้าร์เพศผู้เป็นเวลา 90 วัน พบว่าสารสกัดมะเกี๋ยงที่สกัดด้วยเอธานอลสามารถลดปริมาณมาลอนไดอัลดีไฮด์ (ผลผลิตสุดท้ายของ lipid peroxidation)ในตับแต่ไม่มีผลในซีรั่ม นอกจากนี้ยังเพิ่มการทำงานของเอนไซม์ glutathione peroxidase ในตับหนูอีกด้วย ผลของการวิจัยนี้แสดงให้เห็นว่าสารสกัดมะเกี๋ยงที่สกัดด้วย 95% เอธานอล ประกอบด้วยสารกลุ่มแอนโธซัยยานินปริมาณสูงและมีฤทธิ์ต้านอนุมูลอิสระทั้งในหลอดทดลองและในสัตว์ทดลอง

คำสำคัญ: *Cleistocalyx nervosum*, แอนโธซัยยานิน, ฤทธิ์ต้านอนุมูลอิสระ

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INTRODUCTION

Free radicals play an important role in the mechanisms underlying a number of chronic diseases. They can attack and damage cellular macromolecules, including nucleic acids, lipids, proteins and carbohydrates, leading to altered enzymatic activity, cellular signaling, and vital protein function. Oxidative DNA damage caused by free radicals initiates the multistage carcinogenesis process, starting with DNA mutation and accumulation of genetic events resulting in dysplastic cellular appearance, deregulated cell growth and finally turn to carcinoma.[1] In atherogenesis, reactive oxygen species initiate a radical chain reaction leading to reduced nitric oxide availability, enhancement of proinflammatory cytokines and chemical modification of lipoproteins.[2] In diabetes, free radicals can stimulate glycation of proteins, inactivation of enzymes and alteration in the structure and function of collagen basement and other membranes.[3] However, living organisms can defend themselves via antioxidant systems to counteract reactive radical species. Antioxidant systems include antioxidant enzymes, such as superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase, as well as small molecules including glutathione, uric acid and bilirubin.[1] Epidemiological studies have suggested that vegetables and fruits containing such compounds that have preventative effects for cancer, diabetes, cardiovascular diseases and Alzheimer's disease.[4,5] Plant polyphenols, a large group of natural antioxidants ubiquitous in vegetables and fruits, can ameliorate oxidative stress. They trap and scavenge free radicals, regulate nitric oxide, decrease leukocyte immobilization, induce apoptosis, inhibit cell proliferation and angiogenesis as well as exhibit phytoestrogenic activity.[6]

Anthocyanins, common flavonoids found in plants, have a basic flavylium cationic structure, which can be transformed to quinonoidal bases through proton transfer reactions. The latter form plays an important role in the antioxidant action of anthocyanins.[7] The promoting effect of anthocyanins on ARE-regulated phase II enzyme expression seems to be a critical point in modulating the defense system against oxidative stress. Anthocyanins induce the activation of phase II enzymes through the antioxidant response element pathway against oxidative stress-induced apoptosis.[8] Many studies have suggested that anthocyanins have potent anti-inflammatory, anti-carcinogenic and antioxidant properties.[4,5,7,8]

Cleistocalyx nervosum var. *paniala*, common name Ma-kiang, is a purplish red fruit cultivated in northern Thailand. Its flesh is edible and is used to make juice, wine and jam. The rich purplish red color of

the aqueous extract is characterized by an anthocyanin profile. The most common anthocyanin is cyanidin-3-glucoside. Previous study has demonstrated that the methanol extract of *C. nervosum* seed has antigenotoxic effects against chemical induced mutagenesis.[9] *C. nervosum* extracts can stimulate human lymphocyte activity, which could be useful for modulating immune functions of the body.[10] The aqueous extract of *C. nervosum* has chemopreventive effects on the early stages of rat hepatocarcinogenesis via enhancement of antioxidant enzymes and decreased oxidative stress.[11,12] The present study aimed to investigate the chemical constituents and antioxidant activities of *C. nervosum* using both *in vitro* and *in vivo* models.

MATERIALS AND METHODS

Materials

Diethyl ether, 95% (v/v) ethanol, sodium bicarbonate and trichloroacetic acid were purchased from BDH (England); β-NADPH was purchased from Oriental Yeast Co., Ltd. (Japan); Catechin, gallic acid, glutathione reductase, hemin and thiobarbituric acid were purchased from Sigma-Aldrich Chemical Co. (U.S.A); Folin & Ciocalteu's phenol reagent was purchased from Fluka A.G. (Switzerland); Glutathione (Reduced form) was purchased from Wako (Japan); Potassium chloride was purchased from Carlo-Erba (Italy).

Plant material

Fruits of *C. nervosum* were collected from the Horticulture Research Center Region 1, Office of Agricultural Research and Development, Department of Agriculture, Thailand, in July-August, 2010. The plant was identified and confirmed by comparison with voucher specimens of known identity (Punvittayagul and Taya 1), which are deposited at the Queen Sirikit Botanic Garden, Chiang Mai, Thailand. The pulp was manually removed from the seeds, weighed and stored in a freezer at -20 ^{o}C until extraction.

Preparation of *C. nervosum* extracts

The flesh of *C. nervosum* was ground and extracted with distilled water or 95% (v/v) ethanol. The extracts were centrifuged at 3,000 rpm for 15 min, and filtered through a Whatman no.1 filter paper. The extracts were then evaporated and lyophilized. The crude extracts were stored at -20 ^{o}C until use.

Determination of chemical constituents in *C. nervosum* extracts

The total phenolic compound content in each extract was measured by the method of Singelton *et al*. **(**1999**)**. Extracts were added to Folin-Ciocalteu reagent and 7% (w/v) Na_2CO_3. Then, the solution mixtures were incubated for 15 min at 45 ^{o}C. The absorbance of each sample was measured at

760 nm using a spectrophotometer. Gallic acid was used to calibrate the standard curve and the total phenolic content was expressed as mg of gallic acid equivalents (GAE) per 100 g of fresh weight of the sample. [13]

The total flavonoid content was evaluated using the method described by Moksimovic *et al*. **(**2005**)**. The sample extract was incubated with 5% (w/v) $NaNO_2$ for 10 min before adding 10% (w/v) $AlCl_3.6H_2O$. After the mixture was allowed to stand at room temperature for 10 min, 1 M NaOH was added. The absorbance of the reaction mixture was measured at 532 nm using a spectrophotometer. Catechin was used to calibrate the standard curve and total flavonoids content expressed as mg of catechin equivalents (CE) per 100 g of fresh weight of the sample. [14]

The content of cyanidin-3-glucoside was analyzed by HPLC with an Allure C18 column (250x4.6 mm). The mobile phase was an aqueous 10% formic acid as solvent A and the mixture of acetonitrile: water: formic acid (5:4:1) as solvent B. The gradient profile was run with a flow rate of 1.0 ml/min for 30 min. The detection wavelength was 280 nm.[15]

Determination of *in vitro* antioxidant activity by DPPH free radical-scavenging assay

Different concentrations of each extract (1 mg/ml) were added to 0.3 mM 2,2-diphenyl-1-picrylhydrazyl (DPPH) in MeOH solution in a 96-well microplate and allowed to stand at room temperature for 30 min. The resulting colored solution was measured by spectrophotometer at a wavelength 517 nm. Methanol was used as a negative control while 1-100 mg/ml of ascorbic acid was used as a positive control. The percent of inhibition was calculated using the equation, % Inhibition = $(C - S)/C$ x 100 where C is the net absorbance of the negative control and S is the net absorbance of the sample. Percent inhibition was plotted against concentration and the equation for the straight line was used to obtain the IC_{50} value. A lower IC_{50} value indicates greater antioxidant activity.[16]

Animals

All rats were obtained from the National Laboratory Animal Center, Salaya, Nakhon Pathom, Thailand. Female wistar rats, age 8 weeks old, weight approximately 200 g, were used in acute oral toxicity tests. Male wistar rats, age 4 weeks old, were examined for *in vivo* antioxidant activity. The rats were housed under standard environmental conditions of 24 ^{o}C and 12:12 hr dark-light cycle and allowed free access to water and diet. The experimental protocol was approved by The Animal Ethics Committee of Faculty of Medicine, Chiang Mai University.

Acute toxicity test

The acute oral toxicity test was carried out according to the Organization for Economic Co-operation and Development (OECD) guidelines for Testing of Chemicals number 425.[17] The study was comprised of two groups of 5 female rats. The treated group was received 5000 mg/kg bw of ethanolic extract of *C. nervosum* given once orally. The control group received 10 ml/kg bw of water. The experimental animals were observed for 30 min after treatment, followed by observation for 8 hr and once daily throughout the experiment. All rats were sacrificed using an overdose of diethyl ether after 14 days. The internal organs were observed and weighed.

Antioxidant study in rats

Thirty-two rats were divided into 4 groups. Group 1 received 4 ml/kg bw of distilled water as a vehicle control. Groups 2 to 4 received the ethanolic extract of *C. nervosum* at doses of 100, 300 and 1,000 mg/kg bw for 90 days, respectively. All rats were weighed once a week. The amount of diet and water consumption was measured twice a week. At the end of the study, the rats were anesthetized under diethyl ether and sacrificed. The whole blood was taken from the abdominal vein. The liver, spleen and kidney tissues were removed and weighed. Livers were frozen and stored at -80 ^{o}C.

Determination of lipid peroxidation in liver tissues and serum

The liver homogenate was precipitated in 50% Trichloroacetic acid and centrifuged at 6,000 rpm, 4 ^{o}C for 20 min. Two ml of 0.67% thiobarbituric acid was added to the supernatant and the mixture was heated to 100 ^{o}C for 10 min. The reaction was stopped by placing on ice and adding butanol. After centrifugation at 3,000 rpm for 15 min, the resulting solution was measured spectrophotometrically at 532 nm. The amount of malondialdehyde (MDA), the end-product of lipid peroxidation in thiobarbituric acid reactive substance (TBARS) assay, was quantified.[18]

Serum was mixed with 3 N sulfuric acid and 10% phosphotungstric acid. The mixture was centrifuged at 3,000 rpm for 15 min. The sediment was resuspended in thiobarbituric acid reagent, mixed and heated to 100 ^{o}C for 1 hr in a water bath. The reaction was stopped by placing on ice and adding butanol. After centrifugation at 3,000 rpm for 15 min, the resulting solution was measured spectrophotometrically at 532 nm. The amount of TBARS was quantified and used as an index of lipid peroxidation.[19]

Determination of total glutathione

The liver homogenate was centrifuged at 14,000 rpm for 30 min at 4 ^{o}C. Two hundred µl of supernatant were deproteinized with 5% metaphosphoric acid

and the mixture was centrifuged at 14,000 rpm for 30 min. The 200 µl of reaction mixture was composed of deproteinized supernatant, 10 mM sodium phosphate buffer containing 5 mM EDTA (pH 7.5), 4 mM β-NADPH, 6 U glutathione reductase and 10 mM DTNB. After incubation for 30 min, the resulting solution was read by spectrophotometry at a wavelength of 405 nm using a microplate reader. Glutathione concentration in samples was calculated using a calibration curve and expressed as nmol/mg protein.[20]

Preparation of microsome and cytosol of rat liver

One gram of liver sample was homogenized and centrifuged at 10,000 g for 30 min at 4°C. The resulting supernatant was then centrifuged at 100,000 g for 60 min at 4°C to obtain the cytosolic and microsomal fractions. Total protein concentration in each sample was measured by the Lowry method using a bovine serum albumin calibration curve and expressed as mg protein/ml.[21]

Catalase activity assay

The reaction mixture contained 30 mM H_2O_2, 50 mM phosphate buffer, pH 7.0 and sample for a total volume of 750 µl. Catalase activity was estimate by the decrease in absorbance of H_2O_2 at 240nm.[22]

Glutathione peroxidase activity assay

The reaction mixture contained 0.1 M Tris-EDTA buffer (pH 8.0), 0.1 M GSH, 2 mM β-NADPH, 7 mM *tert*-butylhydroperoxide, 10 U Glutathione reductase and 10 µl of sample. The oxidation of β-NADPH was followed by measuring in the decrease in the absorbance at 340nm.[23]

Glutathione reductase activity assay

The reaction mixture contained 100 mM potassium phosphate buffer (pH 7.0), 1.2 mM GSSG and 1.2 mM β-NADPH. Decrease in the absorbance of β-NADPH at 340 nm was monitored spectrophotometrically.[24]

Heme oxygenase-1 activity assay

The reaction mixture containing 50 mM of the substrate hemin, microsomal fraction and rat liver cytosol as a source of biliverdin reductase, 2 mM glucose-6-phosphate, 0.2 U/ml glucose-6-phosphate dehydrogenase, 0.8 mM NADPH and 100 mM potassium phosphate buffer containing $MgCl_2$, pH 7.4 was incubated at 37 °C for 1 hr. The reaction was stopped with chloroform and the chloroform layer was measured spectrophotometrically. Bilirubin formation was calculated from the difference in absorption between 460 and 530nm.[25]

Statistical analysis

All experiments were conducted independently at least three times. Results were expressed as mean ± SD. Statistical significance of difference between groups was determined by one-way analysis of variance (ANOVA) and post hoc least-significant difference (LSD) test. A *p*- value < 0.05 was regarded as significant.

RESULTS

The amounts of chemical constituents and antioxidant capacity of *C. nervosum* extracts are shown in Table 1. The ethanolic extract presented higher amounts of total phenolic compounds, total flavonoids and cyanidin-3-glucoside when compared to those of the aqueous extract. The DPPH- free radical scavenging activity of aqueous and ethanolic extracts of *C. nervosum* is demonstrated in Figure 1. The ethanolic extract (IC_{50} was 1.68±0.51mg/ml) exhibited higher antioxidant capacity than the aqueous extract (IC_{50} was 5.68±2.17mg/ml). Therefore, the ethanolic extract of *C. nervosum* was further investigated in animal models.

Table 1 Chemical constituents of aqueous and ethanolic extracts of *C. nervosum*

Chemical compound [a]	Aqueous extract	Ethanolic extract
Total phenolic compounds (mg eq GAE/g extract)	9.22±1.67	35.67±6.29
Total Flavonoids (mg eq CE/g extract)	5.23±3.60	13.39±5.73
Cyanidin-3-glucoside (ug/mg extract)	3.37±0.42	25.28±2.28

[a] measured for 3 times.

Data are expressed as mean±SD

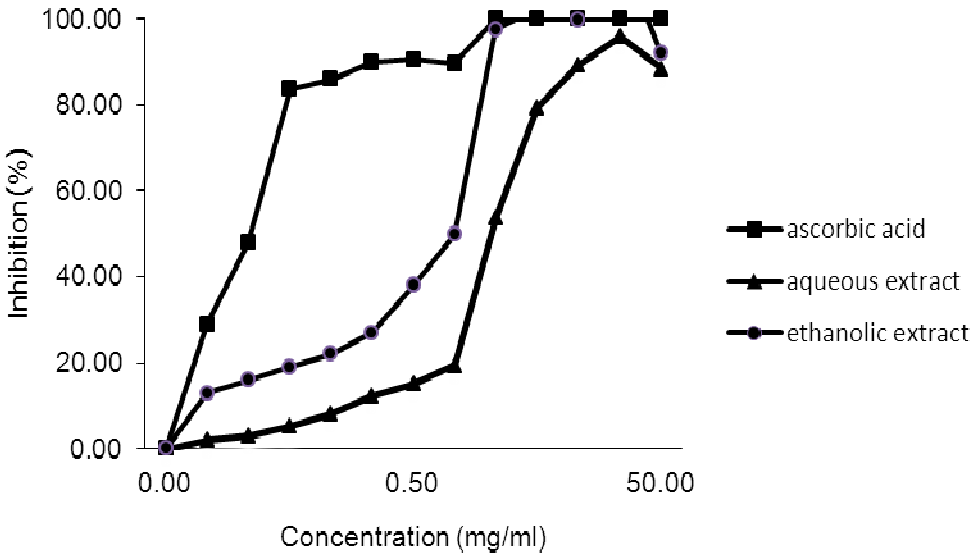

Figure 1 Free radical scavenging of aqueous and ethanolic extracts of *C. nervosum* by the DPPH assay: (□) ascorbic acid; (▲) ethanolic extract of *C. nervosum*; (x) aqueous extract of *C. nervosum*. Results are mean ± SD.

The ethanolic extract of *C. nervosum* did not result in any mortality to rats and no toxic effect was observed throughout the 14 days study period. The body weights, relative organ weights and consumption of diet and water of the treated rats were not significantly different as compared to the control rats (Table 2). In addition, gross examination of internal organs of all rats did not reveal any abnormalities.

The effects of 90 days-administrations of 100, 300 and 1000 mg/kg bw of *C. nervosum* ethanolic extract on oxidative stress and the antioxidant activity in rats are shown in Table 3 and Figure 2. There were no significant differences in body weight change, food and water consumption and vital organ sizes among the treated groups and a vehicle control group (data not shown). The levels of TBARS in liver were significantly decreased (p<0.05) in rats treated with *C. nervosum* ethanolic extract compared to control rats. In contrast *C. nervosum* ethanolic did not affect on TBARS contents in serum. Hepatic glutathione of treated rats was not significantly different when compared to control group. Treatment of 100 and 1000 mg/kg bw the ethanolic *C. nervosum* extract resulted in significant increase in glutathione peroxidase activity (p<0.05), but not of 300 mg/kg bw. While, the ethanolic *C. nervosum* extract did not affect the other antioxidant enzymes including heme oxygenase-1, glutathione reductase and catalase.

Table 2 General observation and relative organ weights of control and *C. nervosum* extract treated rats in the acute toxicity study

Observation	Control (N=5)	5000mg/kg bw of *C. nervosum* ethanolic extract (N=5)
Initial body weight (g)	196±8.94	196±6.52
Final weight (g)	229±8.21	236±4.18
Body weight change (percent)	16.94±4.35	20.52±4.59
Food intake (g/rat/day)	15.83±2.58	16.67±2.04
Water intake (ml/rat/day)	16.67±3.42	21.25±9.45
Heart (g/100g bw)	0.32±0.03	0.32±0.03
Lung (g/100g bw)	0.47±0.04	0.46±0.09
Thymus (g/100g bw)	0.17±0.04	0.17±0.03
Liver (g/100g bw)	3.51±0.24	3.54±0.27
Pancreas (g/100g bw)	0.40±0.09	0.38±0.10
Adrenal gland (mg/100g bw)	30.62±6.52	51.36±10.09
Spleen (g/100g bw)	0.23±0.03	0.26±0.03
Kidneys (g/100g bw)	0.70±0.04	0.67±0.14
Stomach (g/100g bw)	0.46±0.06	0.50±0.05
Ovary (mg/100g bw)	70.04±22.49	75.38±8.37
Fallopian tube (g/100g bw)	0.19±0.06	0.17±0.02

Data are expressed as mean±SD (Number of rats in each group = 5).

Table 3 Effect of ethanolic extract of *C. nervosum* on the levels of serum malondialdehyde and hepatic malondialdehyde, total glutathione and heme oxygenase-1 activity in rats

Treatment	MDA in serum (nmol)	MDA in liver (nmol/mg protein)	Hepatic GSH (nmol/mg protein)	HO-1 (umol/min/mg protein)
Distilled water	4.412±1.74	0.1301±0.004	22.13±3.76	20.70±5.65
C. nervosum 100 mg/kg bw	4.245±1.74	0.0803±0.001*	21.02±5.08	20.79±6.03
C. nervosum 300 mg/kg bw	3.611±1.21	0.0883±0.017*	18.60±4.22	19.03±5.28
C. nervosum 1000 mg/kg bw	4.628±2.86	0.0603±0.001*	17.86±6.01	17.61±3.55

Data are expressed as mean±SD (Number of rats in each group = 8).
MDA = malondialdehyde, GSH = glutathione and HO-1 = heme oxygenase-1.
*Significant difference from control at $p<0.05$.

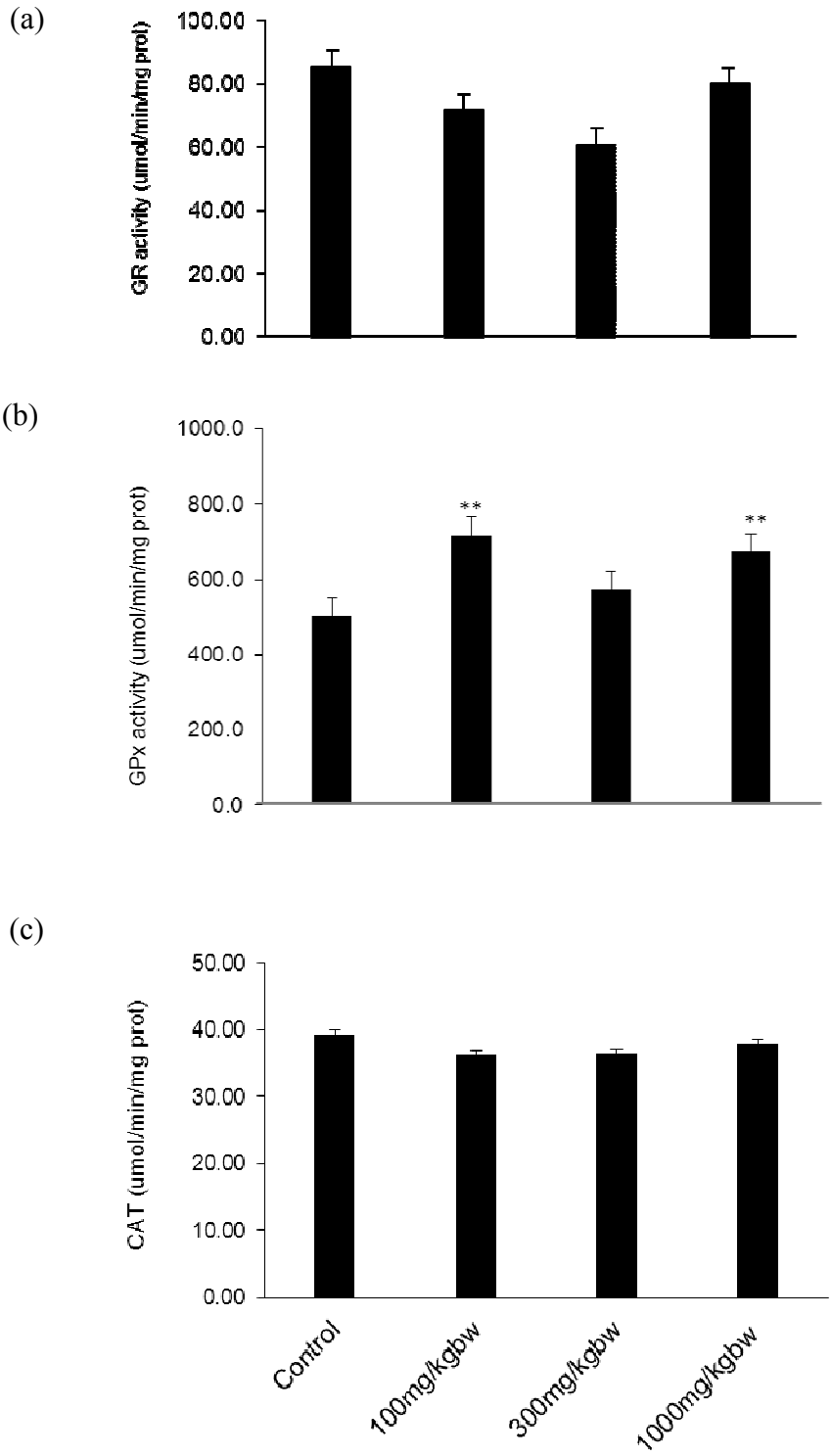

Figure 2 Effect of ethanolic extract of *C. nervosum* on antioxidant enzyme activities in rat livers. (a) glutathione reductase (b) glutathione peroxidase (c) catalase. Results are mean±SD (Number of rats in each group = 8). ***p*<0.05 vs. control

DISCUSSION AND CONCLUSION

The recent study clearly demonstrated that the amounts of polyphenols, flavonoids and cyanidin-3-glucoside as well as the DPPH radical-scavenging capacity were detected at higher level in the ethanolic extract than the aqueous extract of *C. nervosum*. These results support previous findings, that ethanol concentration has a critical role in the extraction of soluble components from different natural products.[26] This may be due to the fact that the *C. nervosum* has more nonpolar chemical constituents than polar ones, thus favoring the less polar solvent. The *in vitro* antioxidant activity was correlated with the content of total phenolic compounds, total flavonoid compounds and anthocyanins. This agrees with previous studies reporting that these compounds may take part in antioxidant capacity involving scavenging of free radicals and/or the chelating iron generating Fenton reaction.[27-28] Glycosylation and hydroxylation of the anthocyanidin backbone affects antioxidant activity. It has been reported that cyanidin is the most common anthocyanidin and the 3-glucoside is the most active antioxidant anthocyanin.[29]

In the experiments involving subchronic treatment with the ethanol extract, the concentrations of TBARS (indicators of lipid peroxide production) were significantly decreased in livers of treated rats while hepatic glutathione peroxidase (GPx) activity was increased. Most tissue damage is considered to be mediated by free radicals attacking membranes through peroxidation of unsaturated fatty acids.[30] The concentration of lipid peroxidation products may reflect the degree of oxidative stress. It has been reported that dietary polyphenols induce the antioxidant/detoxifying enzymes by modulating cellular signaling processes such as Nuclear factor-kappa B (NF-κB) activation, activator protein 1 (AP-1) DNA binding, glutathione biosynthesis, phosphatidylinositol-3-kinase (PI3K)/Akt pathway, Mitogen-Activated Protein Kinase (MAPK) protein activation and the translocation into the nucleus of NF-E2-related factor 2 (Nrf2).[31] GPx is an antioxidant enzyme involved in the detoxification of hydrogen and lipid peroxides and also acts as a peroxynitrite reductase. Shull and his colleagues reported an increase in GPx mRNA at higher concentrations of hydrogen peroxide.[32] Another study suggested a protective role for GPx in diabetes mellitus-associated atherogenesis.[33] In this study, the activities of GPx in rats treated with 100 and 1000 mg/kg bw of the ethanolic extract were enhanced, but not with 300 mg/kg bw. Therefore, the increased activity of GPx might be a part of decreasing of MDA levels in ethanolic extract treated rats. On the other

hand, the antioxidative effect of *C. nervosum* ethanolic extract could be due to its polyphenols with hydroxyl groups, which can act as reducing agents. Ethanolic extract of *C. nervosum* treatment did not affect total glutathione, glutathione reductase, catalase or heme oxygenase-1 in rats. This may suggest that under normal physiological conditions, there is a critical balance in the generation of oxygen free radicals and antioxidant defense systems. Thus, the formation of antioxidant enzymes is related to changes in the levels of free radicals.

In conclusion, the 95% ethanolic extract of *C. nervosum* contains a high content of anthocyanin that showed relatively good antioxidant capacities both in *in vitro* and *in vivo* models. In addition, *C. nervosum* with antioxidant capacities may be acting as naturally occurring antioxidant agents.

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TOTAL PHENOLIC CONTENT, CELLULAR ANTIOXIDANT ACTIVITY AND POTENTIAL HEPATOPROTECTIVE EFFECT OF FRUIT EXTRACTS

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ABSTRACT

Environmental pollutants are sources of several reactive oxygen species and other byproducts of oxidative stress. Such products involve the underlining processes of chronic diseases, either at the initial or progressive stages of the diseases. The antioxidant nutrients and related bioactive compounds common in vegetables and fruits have become a beneficial alternative to prevent oxidative stress in cells. The present study was done to investigate cellular antioxidant activity as well as total phenolic content of the extracts of selected fruits: strawberry, carambola, guava, longkong, pomelo, and tangerine. The protective effect of these fruits on H_2O_2 induced cytotoxicity was also evaluated in human liver carcinoma cell line (HepG2). Cellular antioxidant activity (CAA) assay was the measurement the ability of antioxidants in fruit extracts to prevent oxidation of cell membrane lipids and production of more radicals in cells. The cytoprotective potential was assessed by using MTT assay. The results showed that strawberry, carambola, and guava had higher phenolic contents than longkong, pomelo, and tangerine. The antioxidant efficacies of fruits in CAA values were consistent with their phenolic contents except longkong which exhibited the highest CAA even contained low level of phenolics. However, cell viability measured after co-treatment H_2O_2 with the fruit extracts showed the similar cytoprotective ability of all fruit extracts. The present study demonstrates that all selected fruit extracts have protective effect on H_2O_2 induced oxidative damage in human hepatocarcinoma cells and this effect is related to their cellular antioxidant properties.

Keywords: Phenolic content, Fruit extracts, Cellular antioxidant activity, Hepatoprotective effect

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ปริมาณสารฟีนอลิครวม ฤทธิ์ต้านอนุมูลอิสระในเซลล์และความสามารถในการป้องกันเซลล์ตับของสารสกัดจากผลไม้

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บทคัดย่อ

มลพิษในสิ่งแวดล้อมเป็นแหล่งของสารอนุมูลอิสระของออกซิเจนและสารอื่นๆที่เกิดจากภาวะ oxidative stress ซึ่งเป็นสาเหตุของโรคเรื้อรังชนิดต่างๆหรือทำให้ความรุนแรงของโรคดังกล่าวเพิ่มขึ้น สารอาหารและสารออกฤทธิ์ทางชีวภาพที่พบมากในผักและผลไม้สามารถป้องกันและยับยั้งผลของอนุมูลอิสระในเซลล์ได้ ในการศึกษาครั้งนี้มีการตรวจสอบ ปริมาณสารฟีนอลิครวม ฤทธิ์ต้านอิสระในเซลล์ และความสามารถในการป้องกันการเกิดพิษต่อเซลล์ตับ (HepG2)ซึ่งเหนี่ยวนำโดยสารไฮโดรเจนเปอร์ออกไซด์ของสารสกัดจากสตรอเบอร์รี่ มะเฟือง ฝรั่ง ลองกอง ส้มโอ และส้มเขียวหวาน การวัดฤทธิ์ต้านอิสระในเซลล์เป็นการวัดความสามารถของสารสกัดในการป้องกันการเกิดออกซิเดชั่นของไขมันที่เยื่อหุ้มเซลล์และส่งผลให้มีการสร้างอนุมูลอิสระในเซลล์ ส่วนการป้องกันการเกิดพิษต่อเซลล์ใช้เทคนิค MTT assay ผลการศึกษาพบว่าปริมาณสารฟีนอลิครวมในสารสกัดจากสตรอเบอร์รี่ มะเฟือง และฝรั่งสูงกว่าในลองกอง ส้มโอ และส้มเขียวหวาน ประสิทธิภาพของการต้านอนุมูลอิสระในเซลล์ของสารสกัดจากผลไม้มีความสัมพันธ์กับปริมาณสารฟีนอลิครวม ยกเว้นลองกองซึ่งแสดงฤทธิ์ต้านอนุมูลอิสระในเซลล์ได้สูงสุดทั้งๆที่มีปริมาณสารฟีนอลิครวมต่ำ อย่างไรก็ตามจากการให้สารสกัดจากผลไม้ร่วมไปกับการให้สารไฮโดรเจนเปอร์ออกไซด์เพื่อเหนี่ยวนำให้เกิดพิษกับเซลล์ตับ พบว่าสารสกัดจากผลไม้ทุกชนิดมีประสิทธิภาพในการป้องกันหรือลดพิษจาก oxidative stress ไม่แตกต่างกัน การศึกษานี้แสดงให้เห็นว่าสารสกัดจากผลไม้ที่เลือกมาทุกชนิดสามารถป้องกันความเสียหายจากความเป็นพิษของสารไฮโดรเจนเปอร์ออกไซด์ในเซลล์ตับได้ ซึ่งผลดังกล่าวเนื่องมาจากความสามารถต้านอนุมูลอิสระในเซลล์ของสารสกัด

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INTRODUCTION

Oxidants and byproducts found in environmental pollutants have the capability to produce reactive radicals in biological systems and lead to oxidative stress, a state of the imbalance between the excessive formation of reactive oxygen species (ROS) and insufficient body antioxidant protection. The oxidative stress can initiate and implicate in several human chronic diseases such as cancer, cardiovascular disease, diabetes, atherosclerosis, neurological disorders, and other chronic inflammatory diseases.[1, 2] Oxidative stress also plays an important role in the induction and progression of toxic liver diseases and other hepatic alterations.[3,4] Consumption of fruits and vegetables has been negatively associated with incidence and mortality rates caused by cancer, hypertension, cardio- and cerebrovascular diseases in several human studies. Dietary components in fruits and vegetables may act independently or in combination as natural antioxidants that have been shown to play a crucial role in prevention of such diseases.[5]The constituents of fruits and vegetables that act as antioxidants include vitamins such as vitamin C, vitamin E, β-carotene, and polyphenolic compounds.[6] Phenolic compounds possess antioxidant properties through free-radical scavenging activity resulted from their hydrogen- or electron-donating ability, their metal chelating properties, as well as the stability of the resulting antioxidant-derived radicals.[7, 8] It has been demonstrated that antioxidant activities of fruits show high correlations with levels of their total soluble phenolic contents.[9] However, chemical antioxidant activity does not account for bioavailability, uptake, and metabolism of the antioxidant compounds. Cell culture models can provide the tool for investigation of uptake, distribution, and metabolism. Therefore, a cell-based antioxidant activity assay was developed to evaluate foods, phytochemicals, and dietary supplements for potential biological activity.[10, 11]

In the present study, total phenolic contents and cellular antioxidant activities of the selected fruit extracts were determined. Furthermore, the antioxidative effect of the fruit extracts were confirmed by assessing their cytoprotective potential against hydrogen peroxide (H_2O_2) induced oxidative damage in human hepatic cell line (HepG2).

MATERIALS AND METHODS

Chemicals

2′,7′-Dichlorofluorescin diacetate (DCFH-DA), Folin Ciocalteu reagent, gallic acid and sodium carbonate were purchased from Sigma-Aldrich, Inc. (St. Louis, USA). 2,2′-azobis (2-amidinopropane) dihydrochloride (ABAP) was purchased from Wako Chemicals USA. Methanol and acetone were bought from Mallinckrodt

Baker, Inc. (Phillipsburg, USA). Dulbecco's Modified Eagle Medium (DMEM), Hanks' Balanced Salt Solution (HBSS) and fetal bovine serum (FBS) were purchased from Life Technologies (Carlsbad, USA). HepG2 human hepatocarcinoma cells were obtained from the American Type Culture Collection (ATCC) (Rockville, USA).

Preparation of Fruit Extracts

Strawberry, carambola, guava (Pan Sitong), longkong, pomelo (Thong Dee), and tangerine (Sai Nam Pung) were collected from three venders of local markets. Two hundred grams of edible portions of each kind of fruit from each vender were homogenized to make the pooled sample. The preparation of fruit extract was performed by modified method of Sun et al. (2002).[12] One hundred gram of each pooled fruit sample was extracted by stirring in chilled 80% acetone (1:2, w/v) at 5-8 °C for 6 hrs. After filtration through Whatman no. 1 paper, the solvent in extracts were evaporated under vacuum at room temperature until approximately 10% of original volumes remain. The concentrated extracts were reconstituted to 50 ml in 70% methanol and stored at -30°C. The methanol was evaporated under a stream of nitrogen, and the extracts were reconstituted to the same volume in water before use.

Cell Culture

HepG2 cells were grown in DMEM media supplemented with 10% FBS and 1% penicillin/streptomycin. Cells were maintained in humidified incubator at 37ºC and 5% CO_2.

Determination of Total Phenolic Content

The total phenolic contents of the fruits were measured using a modified colorimetric Folin Ciocalteu method. [13] Briefly, fruit extracts were diluted with de-ionized water and introduced to a test tube. Then, 0.125 ml of Folin Ciocalteu reagent was added to the solution and allowed to react for 6 min. A 1.25 ml aliquot of 7% sodium carbonate solution was added into the test tubes. The mixture was then diluted to 3 ml with deionized water and allowed to stand for 90 min to develop color. Absorbance at 765 nm was measured spectrophotometrically. The measurement was compared to a standard curve of gallic acid concentrations and expressed as milligrams of gallic acid equivalents (GAE) per 100g of fresh weight.

Cellular Antioxidant Activity (CAA) of Fruit Extracts

The CAA assay was carried out according to the method of Wolfe et al. (2007).[10] Briefly, HepG2 cells were seeded at a density of 6×10^4 cells/ well on a 96-well microplate. The growth medium was

removed after 24 h, and the cells were washed with PBS. Cells were treated in triplicate for 1 h with treatment medium containing various concentrations of tested fruit extracts plus 25 μM DCFH-DA. Dye was removed and cells were washed with PBS. Then 600μM ABAP in HBSS was applied to the cells and the 96- well microplate was placed into a microplate reader (Wallac 1420, Finland) at 37 °C. Emission at 538 nm was measured after excitation at 485 nm every 5 min for 1 h.

After blank subtraction, the area under the curve for fluorescence versus time was integrated to calculate the CAA value at each concentration of fruit as

CAA unit = 1 - (∫SA/∫CA)

∫SA is the integrated area under the sample fluorescence versus time curve

∫CA is the integrated area from the control curve

The median effect plot of log (fa/fu) versus log (dose) was done based on calculated CAA unit.

fa is the fraction affected (CAA unit) by the treatment.

fu is the fraction unaffected (1 - CAA unit) by the treatment.

The EC50 is then determined as concentration of extract at which fa/fu = 1 (i.e., CAA unit 50), as calculated from the linear regression of the median effect curve.

Protective Effect on H_2O_2 Induced Oxidative Damage in HepG2 Cells

HepG2 cells were seeded at $1x10^4$ cells/ well on a 96-well plate and incubated at 37 °C for 24 h. After removing medium, and washing cells with PBS, 200 μM H_2O_2 with or without different concentration of fruit extracts in 100 μl of HBSS were applied to the cells, then the plate was incubated at 37 °C for 24 h. After treatments, the cell monolayer was washed once with PBS, added with a 0.5 mg/ml MTT solution in DMEM and incubated at 37 °C for 2 h. Afterwards, the medium with unreacted MTT was removed, and then isopropanol/ HCL solution was added to dissolve the reduced dye. The absorbance was measured at 570 nm with the microplate reader (BioTek, USA). The viability of the treated groups was assessed as a percentage of non-treated control groups, which was assumed to be 100%.

Statistical analysis

All values were presented as mean± standard error of mean (SEM). Statistical analysis of the data for multiple comparisons was performed by one-way analysis of variance (ANOVA) followed by Multiple Comparison Test for pair wise comparison. A level of $P<0.05$ was accepted as statistical significant.

RESULTS AND DISCUSSION

Phenolic Content of the Fruit Extracts

The phenolic compounds have been established as the main contributors to the antioxidant activity of fruits and vegetables**.** They act as powerful antioxidants in a structure-dependent manner by scavenging reactive oxygen species (ROS), and chelating transition metals which play vital roles in the free radical reactions.[14] The redox properties can promote phenolic compounds to act as reducing agents, hydrogen donators and singlet oxygen quenchers.[5] Table 1 shows total phenolic contents (TPC) that categorized fruits into the high and low TPC groups. Strawberry, guava and carambola contained the high TPC of 272, 176, and 137 mgGAE/100g FW, respectively.

The low TPC values in longkong, pomelo and tangerine were in the range of 53-64 mgGAE/100g FW. These results are consistent with those observed in a previous study, in which TPC of guava and carambola extracts were 4-5 folds higher than TPC in longkong and tangerine extracts.[15] Isabelle et al. (2010) also investigated the TPC of fruits in Singapore and reported the TPC values of strawberry, guava, carambola, pomelo and tangerine that were similar to our findings.[16] This may result from most of the tropical fruits in Singapore are imported from South East Asian countries including Thailand.

Table 1 Total phenolic content and cellular antioxidant activity of selected fruits expressed as EC50 values (mean ± SEM, n =3)

Fruits	Total phenolic content (mg GAE /100 g FW)	EC50 (mg/mL)
Strawberry	272.87± 3.56	5.75
Guava	176.48±.2.82	6.31
Carambola	137.82± 2.62	7.50
Longkong	64.21± 2.95	4.37
Pomelo	55.18± 5.48	55.95
Tangerine	53.10± 6.76	27.54

Cellular Antioxidant Activity (CAA)

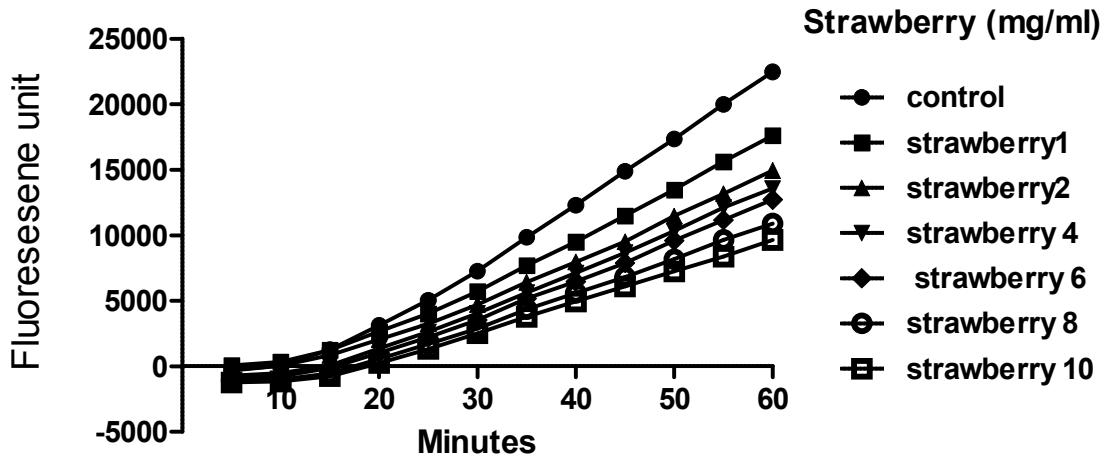
The antioxidant potential of selected fruits was evaluated by measuring their ability to prevent ABAP induced oxidation of non-fluorescent dichlorofluorescin diacetate (DCFH-DA) to fluorescent dichlorofluorescin (DCF) in HepG2 cells. The example of dose dependent inhibition of ABAP induced increase in fluorescence by strawberry and longkong extracts were illustrated in Figure 1A. and 2A. The median effect plot generated using the data of strawberry and longkong extracts were shown in Figure 1B. and 2B.

In this study, the cellular antioxidant activities of selected fruits were determined and expressed by the EC50 values that represented the concentration of fruit extracts which inhibited 50% of intracellular ROS generation. In general, the lower EC50 values indicated the higher anti-free radical efficacy (CAA) of the extracts.

The EC50 values shown in Table 1 demonstrate that longkong extract has the lowest EC50 value, followed by strawberry, guava, and carambola which have similar EC50 values. Tangerine and pomelo extracts show the much higher EC50 values.

Therefore, of the fruit tested, longkong, strawberry, guava, and carambola exhibited the much high antioxidative activities in HepG2 cells compared to tangerine and pomelo. Except longkong, all fruits with the high CAA showed the correlation between the higher TPC and the lower EC50 values. From this point of view, the phenolic compounds in strawberry, guava and carambola may directly contribute to their cellular antioxidant action. In contrast, longkong which was the fruit had the highest CAA as well as tangerine or pomelo which presented the low CAA, contained the similar low level of phenolic contents. Tangerine possessed higher CAA than pomelo in our study had been reported to contain 7- and 10 fold, respectively, higher levels of beta-carotene and lycopene than pomelo. However, longkong which showed the highest CAA but low in TPC in this study did not contain beta-carotene and lycopene in the detected level.[17] These results suggested that the CAA of later fruit group may be contributed from other non-phenolic antioxidants. Moreover, the bioavailability and potency of antioxidants presented in each fruit are important factors influencing antioxidative potential in cells.

A



B

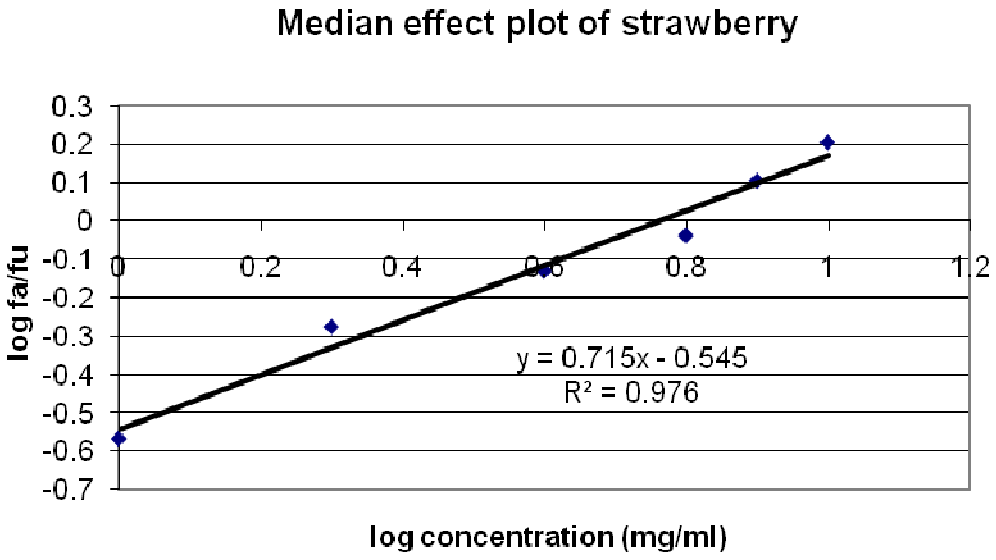
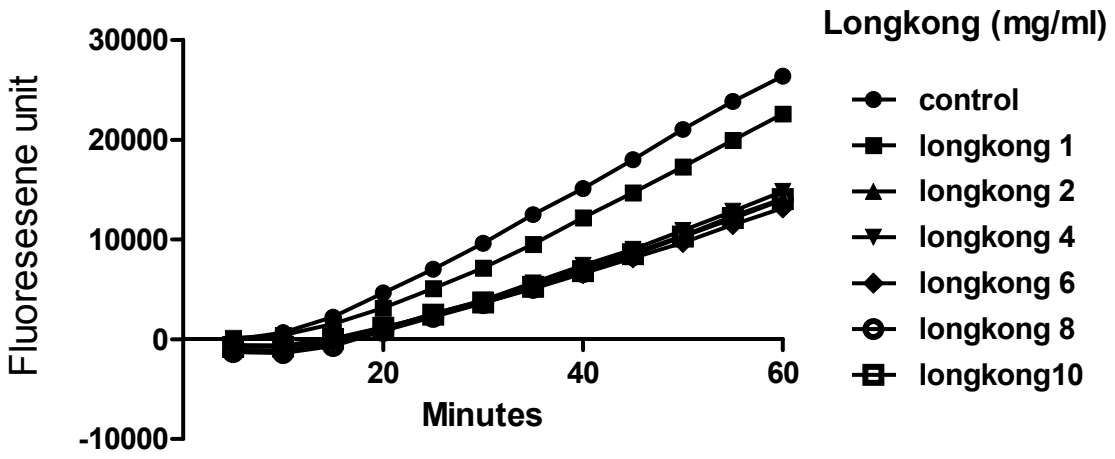


Figure 1 Cellular antioxidant activity assay results. (A) Peroxyl radical-induced oxidation of DCFH to DCF in HepG2 cells and the inhibition of oxidation by strawberry (B) Median effect plots for inhibition of peroxyl radical-induced DCFH oxidation by strawberry (mean ± SEM, n =3)

A



B

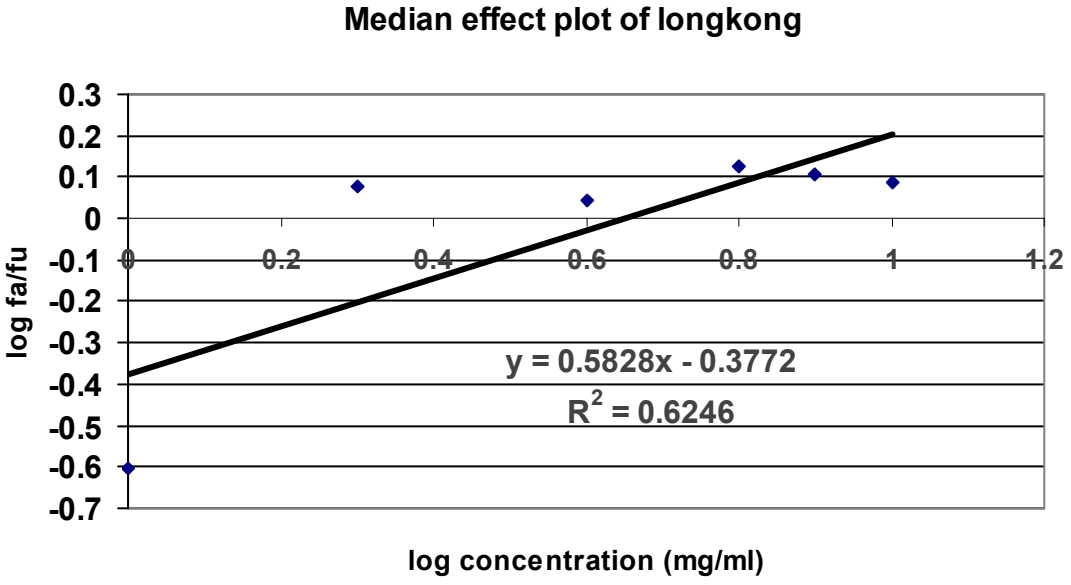


Figure 2 Cellular antioxidant activity assay results. (A) Peroxyl radical-induced oxidation of DCFH to DCF in HepG2 cells and the inhibition of oxidation by longkong (B) Median effect plots for inhibition of peroxyl radical-induced DCFH oxidation by longkong (mean ± SEM, n =3)

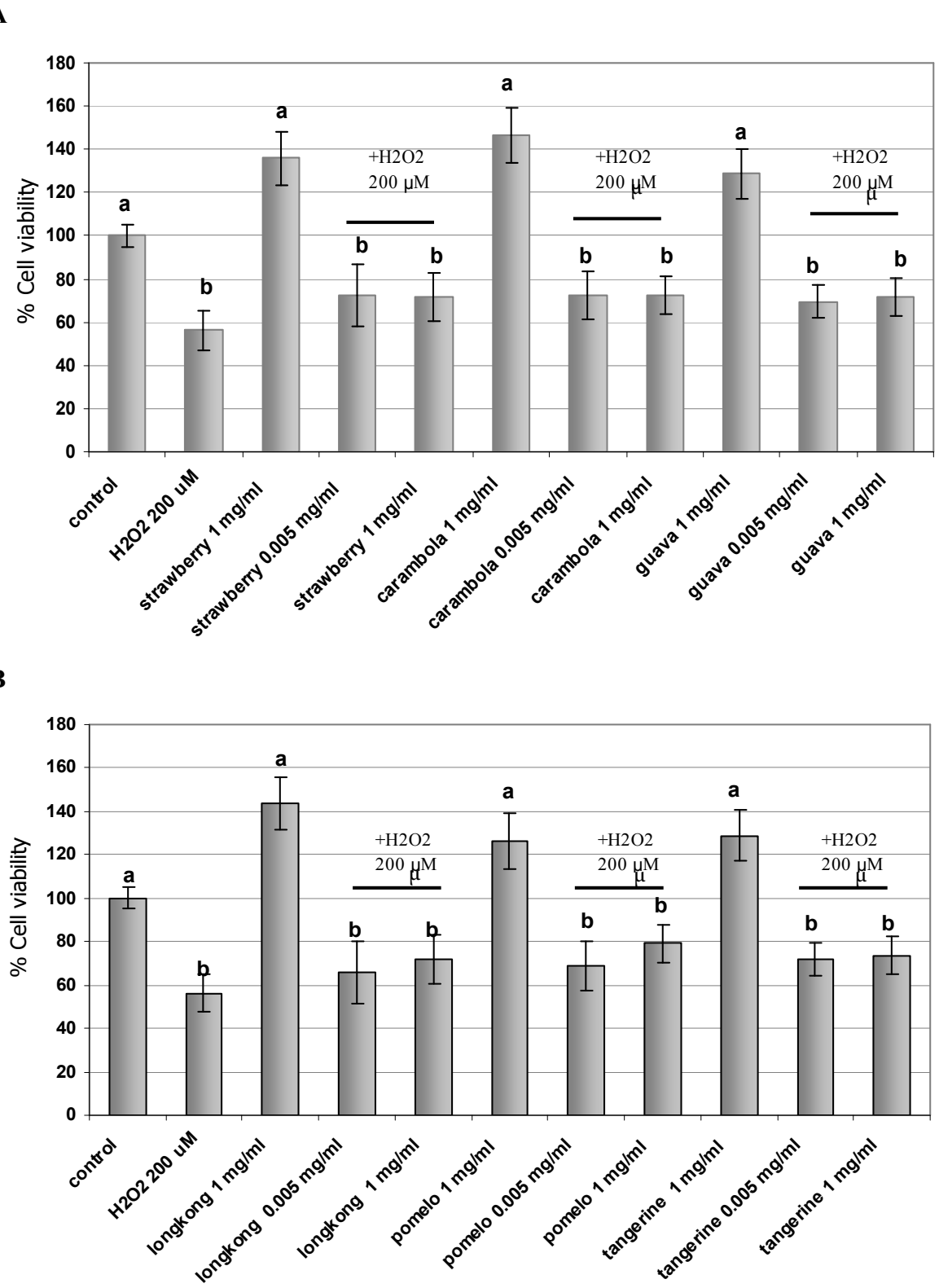


Figure 3 Effect of fruit extracts on H_2O_2-induced oxidative damage in HepG2 cells. (A) Cells incubated in 200 µM H_2O_2 with or without 0.005, 1 mg/mL of strawberry, carambola, and guava extracts for 24 h. (B) Cells incubated in 200 µM H_2O_2 with or without 0.005, 1 mg/mL of longkong, pomelo, and tangerine extracts for 24 h. (mean ± SEM, n =6**)**

Protective effect on oxidative damage in HepG2 cells

H_2O_2 generated from sources of oxidative stress and oxygen radicals can form highly reactive radicals such as hydroxyl radicals in the presence of transition metal ions or by various other mechanisms. The formation of hydroxyl radicals and other ROS initiates oxidation of major cellular constituents such as lipid, DNA and protein and induces oxidative stressed cell damage.[18] Therefore, protective effect of strawberry, guava, carambola, longkong, pomelo, and tangerine extracts against H_2O_2-induced cell death was investigated in this study. As shown in Figure 3A and 3B, all fruit extracts at the maximum concentration used 1mg/ml increased the cell viability up to about 120-140% as compared to control. It was indicated that the fruit extracts at the level up to 1mg/ml was not toxic to HepG2 cells. The addition of 0.005 and 1 mg/ml of fruit extracts simultaneously with H_2O_2 increase the cell viability to 65-80% as compared to 56% in H_2O_2-treated cells. These results suggest that co-administration of all fruit extracts with H_2O_2 tends to improve cell survival rate although not to a level that is statistically significant. It was reported that strawberry, carambola and guava which presented the high CAA in this study also had the high antioxidant activities evaluated by oxygen radical scavenging activity (ORAC) and 2,2-diphenyl-1-picrylhydrazyl radical scavenging assay (DPPH). [9, 15, 19] However, the protective ability against H_2O_2-induced oxidative cell damage of all six selected fruits was not different. This result is probably due to by co-administration of H_2O_2 with the fruit extracts, the antioxidants in extracts can react directly with induced free radical before entry the cells. In addition, the doses of fruit extracts used may release the mixed antioxidants which can neutralize and reduce free radicals to the similar levels.

CONCLUSION

Overall, the data from this study has demonstrated the cellular antioxidant activity of fruits which account for total antioxidant contents in fruits and cellular mechanism. Screening approaches by using CAA assay may obtain the more databases associate with potential biological activity that is not found from the chemistry antioxidant activity assays. Consumption of all tested fruit varieties may deliver healthful benefits by supplying natural antioxidants that are protective against oxidative hepatic cellular damage.

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SIMPLE ASPHYXIATION DUE TO ACCIDENTAL ARGON GAS INHALATION IN A COATING WORKER: A CASE REPORT

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ABSTRACT

Argon is an inert gas mostly used as shielding gas in metal welding processes. Although argon is non-toxic, it does not satisfy the body's need for oxygen and is thus a simple asphyxiant. By this mechanism, argon is considered highly dangerous especially within closed areas. When argon available in confined spaces, it can cause hypoxia and even death.

This report described the clinical presentation and clinical course of a coating worker who suffered from argon inhalation by incorrectly used of a self-contained breathing apparatus (SCBA). The patient faulty connected his airline respirator to argon source instead of oxygen during his work. He was found to have alteration of conscious and hypoxic symptoms. After emergency transferred to a nearby hospital and treated with oxygen he was survived.

Keywords: Argon, Inert gas, Asphyxiant, Air-line respirator

ภาวะพิษจากอาร์กอนแทนที่ออกซิเจนในอากาศในคนงานพ่นสีท่อเหล็ก

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เรื่องย่อ

อาร์กอนเป็นแก๊สเฉื่อยที่นิยมใช้อย่างแพร่หลายในอุตสาหกรรมเชื่อมโลหะ เนื่องจากอาร์กอนเป็นแก๊สที่ไม่ทำปฏิกิริยา โดยธรรมชาติอาร์กอนไม่มีพิษในตัวเอง แต่สามารถก่ออันตรายโดยการทำให้ร่างกายเกิดภาวะขาดออกซิเจนได้ ด้วยการเข้าไปแทนที่ออกซิเจนในอากาศโดยเฉพาะอย่างยิ่งในภาวะอับอากาศ ซึ่งสามารถทำให้ร่างกายขาดออกซิเจนจนกระทั่งเสียชีวิตได้

รายงานฉบับนี้บรรยายถึงอาการแสดงและการดำเนินโรคของคนงานแผนกพ่นสีรายหนึ่ง ซึ่งได้รับแก๊สอาร์กอนจากความผิดพลาดในการต่อท่ออากาศเข้าสู่เครื่องช่วยหายใจแบบครอบศีรษะ โดยเชื่อมต่อแก๊สอาร์กอนแทนที่จะเป็นแก๊สออกซิเจน ทำให้ร่างกายได้รับอาร์กอนที่มีความเข้มข้นสูงภายในที่ครอบศีรษะ อาร์กอนไปแทนที่ออกซิเจนในอากาศ ทำให้ระดับออกซิเจนในอากาศที่หายใจลดต่ำ และร่างกายเกิดภาวะขาดออกซิเจนขึ้น คนงานรายนี้มาโรงพยาบาลด้วยอาการความรู้สึกตัวลดลง สับสน อาเจียน หลังจากได้รับแก๊สอาร์กอนขณะกำลังทำงานพ่นสีท่อเหล็กภายในโรงงาน หลังจากการรักษาด้วยการให้ออกซิเจน คนงานรายนี้สามารถรอดชีวิตได้ในที่สุด

คำสำคัญ: อาร์กอน แก๊สเฉื่อย กลุ่มของแก๊สที่ทำให้เกิดภาวะขาดออกซิเจน ท่ออากาศ

บทนำ

อาร์กอน (Argon; สัญลักษณ์อะตอม Ar) เป็นแก๊สเฉื่อย (inert gas) ชนิดหนึ่ง โดยธรรมชาติมีประกอบอยู่ในบรรยากาศโลกประมาณ 0.93 % แก๊สเฉื่อยเป็นกลุ่มแก๊สหายาก (rare gas) ซึ่งมีเสถียรภาพและมีอัตราการเกิดปฏิกิริยากับธาตุอื่นๆ ต่ำมาก หรือไม่ทำปฏิกิริยาเลย ซึ่งอาจเรียกได้อีกอย่างว่า แก๊สมีตระกูล (noble gas) กลุ่มแก๊สเหล่านี้ได้แก่ ฮีเลียม (He) นีออน (Neon) อาร์กอน (Ar) คริปทอน (Kr) ซีนอน (Xe) และเรดอน (Rn)

อาร์กอน ไม่มีสี ไม่มีกลิ่น ไม่มีรส ไม่กัดกร่อน ไม่ติดไฟ และไม่มีพิษในตัวเอง[1] มีเลขอะตอม 18 ความถ่วงจำเพาะเท่ากับ 1.38 ซึ่งหนักกว่าอากาศ อาร์กอนจะกลายเป็นของเหลวที่อุณหภูมิ -186 องศาเซลเซียส นอกจากนี้อาร์กอนยังเป็นนำความร้อนได้ต่ำและละลายในน้ำได้เพียงเล็กน้อย

เนื่องจากมีราคาถูก อาร์กอนจึงได้รับความนิยมใช้อย่างแพร่หลายในอุตสาหกรรมเชื่อมโลหะ (metal welding) ซึ่งจะใช้อาร์กอนเป็นแก๊สปกคลุมในระหว่างการเชื่อม เพื่อป้องกันไม่ให้ชิ้นงานสัมผัสกับอากาศ ซึ่งชิ้นงานเหล่านี้อาจทำปฏิกิริยากับไนโตรเจนหรือออกซิเจนในอากาศ ทำให้เกิดสนิมที่ผิวงานเชื่อม ทำให้รอยเชื่อมไม่แข็งแรง อาร์กอนจะทำหน้าที่ปกคลุมบริเวณที่เชื่อมเพื่อป้องกันการเกิดปัญหา ช่วยให้การเชื่อมติดสนิทดี เกิดสนิมตรงรอยเชื่อมลดลง ชิ้นงานมีคุณภาพและมีความเสถียร โดยอาร์กอนถูกนำไปใช้ในกระบวนการเชื่อมทั้งแบบ Gas Metal Arc Welding (GMAW) และ Gas Tungsten Arc Welding (GTAW) นอกจากใช้ในงานเชื่อมแล้ว อาร์กอนยังถูกใช้ในอุตสาหกรรมการสังเคราะห์สารที่ต้องใช้อุณหภูมิสูง (high-temperature industrial processes) เช่น การเผาหลอมกราไฟต์ต้องทำในบรรยากาศของอาร์กอน เนื่องจากอาร์กอนสามารถคงสมบัติการไม่ทำปฏิกิริยากับสารเคมีอื่นได้แม้อยู่ในสภาวะที่มีอุณหภูมิสูงมาก

โดยทั่วไปอาร์กอนจะไม่ทำปฏิกิริยาหรือก่ออาการพิษใดๆ ให้กับร่างกาย แต่ในกรณีที่ได้รับเข้าไปในปริมาณมาก ความเข้มข้นสูง ก็สามารถก่ออันตรายให้กับมนุษย์ได้ เพราะอาร์กอนจะทำตัวเป็น simple asphyxiant แก๊สในกลุ่ม simple asphyxiant นี้หมายถึง แก๊สใดก็ตามที่ไม่มีพิษในตัวเอง แต่สามารถเข้าไปแทนที่ออกซิเจนในอากาศ ทำให้อากาศที่หายใจเข้าไปมีความเข้มข้นของออกซิเจนน้อยลง เกิดความผิดปกติขึ้นเนื่องจากการขาดออกซิเจนได้ โดยไม่ทำให้เกิดภาวะพิษต่อเซลล์ ซึ่งเป็นข้อแตกต่างจาก systemic asphyxiant (อาจเรียก toxic asphyxiant หรือ chemical asphyxiant ก็ได้) ซึ่งจะมีพิษต่อเซลล์ได้ ทำให้เซลล์ขาดออกซิเจน แม้ว่าอากาศที่หายใจเข้าไปจะมีระดับความเข้มข้นของออกซิเจนปกติ[2]

บทความนี้รายงานลักษณะทางคลินิกของการสูดดมแก๊สอาร์กอน รวมทั้งสาเหตุของการได้รับแก๊สนี้

รายงานผู้ป่วย

ผู้ป่วยชายไทยคู่ อายุ 30 ปี ไม่มีโรคประจำตัว ไม่สูบบุหรี่ ถูกนำส่งโรงพยาบาลด้วยอาการความรู้สึกตัวลดลง สับสน อาเจียน หลังจากสูดดมแก๊สอาร์กอนนานประมาณ 2 นาที ขณะทำงานพ่นสีท่อเหล็กภายในโรงงาน

ผู้ป่วยทำงานเป็นพนักงานประจำอยู่ในโรงงานผลิตท่อเหล็ก ซึ่งเป็นท่อที่ใช้สำหรับการ

ขนส่งน้ำ น้ำมัน และแก๊สธรรมชาติ ตามแท่นขุดเจาะแก๊สธรรมชาติในทะเล ผู้ป่วยมีหน้าที่ประจำคือการพ่นสีท่อเหล็ก โดยกระบวนการพ่นสีท่อเหล็กนั้น ทางโรงงานจะให้พนักงานทำในห้องพ่นสี ให้ใส่ชุดกันสารเคมีอย่างรัดกุม และใส่อุปกรณ์ช่วยหายใจ (breathing apparatus) ที่มีลักษณะเป็นที่ครอบศีรษะต่อกับสายออกซิเจน ซึ่งต่อมาจากวาล์วจ่ายออกซิเจน วัตถุประสงค์ที่ให้ใช้อุปกรณ์ช่วยหายใจชนิดนี้ เพื่อให้พนักงานลดการสัมผัสไอระเหยของสีพ่นในระหว่างการทำงาน การดำเนินการจะใช้ระบบมีเพื่อนคอยดูแล (buddy) คือจะมีคนที่ทำหน้าที่พ่นสีคนหนึ่ง และมีคนคอยดูแลช่วยเหลือคนที่พ่นสีอยู่อีกอย่างน้อย 2 คน รวมทั้งทีมเป็น 3 คน แผนกพ่นสีนี้ ทั้งแผนกมีพนักงานรวมทั้งหมด 50 คน จะหัวหน้างานดูแลโดยรวมคนหนึ่ง

เหตุการณ์เกิดในวันที่ 23 กุมภาพันธ์ พ.ศ. 2555 เวลาประมาณ 20.00 น. ผู้ป่วยได้ใส่ชุดปฏิบัติงานเพื่อจะเริ่มดำเนินการพ่นสี ผู้ป่วยได้นำสายออกซิเจนซึ่งต่อจากครอบศีรษะของตนเองไปต่อเข้ากับวาล์วจ่ายแก๊สผิดชนิด โดยแทนที่จะต่อเข้ากับวาล์วจ่ายแก๊สออกซิเจน แต่ผู้ป่วยนำไปต่อเข้ากับวาล์วจ่ายแก๊สอาร์กอนแทน *สอบถามในภายหลังผู้ป่วยให้ข้อมูลว่า สาเหตุที่ตนเองต่อสายผิดเนื่องจากวันนั้นเป็นการใช้ชุดอุปกรณ์ช่วยหายใจชุดใหม่ ทำให้ไม่คุ้นเคย อีกทั้งบริเวณที่วาล์วจ่ายแก๊สทั้งสองตั้งอยู่ยังค่อนข้างมืดอีกด้วย สาเหตุที่วาล์วจ่ายแก๊สออกซิเจนและวาล์วจ่ายแก๊สอาร์กอนมาอยู่ใกล้เคียงกัน เนื่องจากภายในโรงงานแห่งนี้มีการดำเนินกิจกรรมการเชื่อมเหล็กและพ่นสีอยู่เป็นจำนวนมาก ทางโรงงานจึงได้เดินท่อส่งแก๊สออกซิเจน (ซึ่งใช้ในการต่อเข้ากับอุปกรณ์ช่วยหายใจ) และแก๊สอาร์กอน (ซึ่งใช้ในงานเชื่อม) กระจายไปทั่วบริเวณโรงงาน และจัดทำวาล์วจ่ายแก๊สไว้ในจุดต่างๆ เพื่อให้พนักงานแต่ละกลุ่มใช้ได้อย่างสะดวก*

หลังจากเปิดแก๊สและเริ่มทำงานพ่นสีไปเพียงประมาณ 2 นาที ผู้ป่วยก็ล้มลง หมดสติ ตัวเกร็งทั้งร่างกาย มีอาการสับสน ไม่มีอุจจาระปัสสาวะราด ไม่มีชักกระตุก เพื่อนที่เป็นผู้ดูแลได้รีบเข้าไปช่วยเหลือ แจ้งหัวหน้างาน และพาไปส่งที่คลินิกหน้านิคมอุตสาหกรรม ระหว่างนำส่งผู้ป่วยมีอาการคลื่นไส้ อาเจียนออกมา 1 ครั้ง ตัวเกร็ง และดิ้นถีบขาไปมา

เมื่อมาถึงคลินิกหน้านิคมอุตสาหกรรมในอีกประมาณ 15 นาทีต่อมา แพทย์ตรวจประเมิน Glasgow coma scale ได้ 11 คะแนน (E=4, V=2, M=5) ผู้ป่วยไม่รู้สึกตัว ดิ้นถีบขาไปมา ความดันโลหิต 120/70 มิลลิเมตรปรอท ชีพจร 90 ครั้งต่อนาที หายใจเร็ว อัตราการหายใจ 20 ครั้งต่อนาที ระดับความอิ่มตัวของออกซิเจนในเลือด (oxygen saturation) แรกรับเท่ากับ 70 % แพทย์ได้ทำการรักษาเบื้องต้นโดยการให้ออกซิเจนบริสุทธิ์ทางหน้ากากและถุงออกซิเจน (oxygen mask with bag) และให้สารน้ำเป็นน้ำเกลือความเข้มข้น 0.9 % (normal saline solution) เมื่อเวลาผ่านไประยะหนึ่ง ระดับความอิ่มตัวของออกซิเจนในเลือดเพิ่มขึ้นมาเป็น 99 % จึงได้ส่งตัวผู้ป่วยมารักษาต่อที่โรงพยาบาล

ผู้ป่วยถูกส่งตัวมารักษาต่อที่โรงพยาบาลแห่งหนึ่ง ในเวลาประมาณ 22.30 น. ที่แผนกฉุกเฉิน ผู้ป่วยยังมีอาการสับสน ดิ้นถีบแขนขาไปมา ตัวเกร็ง และคลื่นไส้ ระดับความอิ่มตัวของออกซิเจนในเลือดเท่ากับ 100 % และประเมิน Glasgow coma score ได้ 9 คะแนน (E=2, V=2, M=5) แพทย์

ประเมินอาการแล้วให้ผู้ป่วยเข้าพักรักษาตัวในหอผู้ป่วยวิกฤต

ครึ่งชั่วโมงต่อมาผู้ป่วยดิ้นถีบน้อยลง ตั้งแต่เริ่มเกิดอาการ ผู้ป่วยไม่มีอาการชักเกร็งกระตุกเลย สามารถหายใจได้เอง โดยได้รับออกซิเจนบริสุทธิ์ทางสายที่ต่อผ่านหน้ากากและถุงออกซิเจน ภาพรังสีทรวงอกปกติ ระดับน้ำตาลในเลือดเท่ากับ 102 มิลลิกรัมต่อเดซิลิตร การทำงานของไตอยู่ในเกณฑ์ปกติ โดยระดับ blood urea nitrogen (BUN) เท่ากับ 10.0 มิลลิกรัมต่อเดซิลิตร และระดับ creatinine (Cr) เท่ากับ 1.2 มิลลิกรัมต่อเดซิลิตร ระดับเกลือแร่พบโพแทสเซียมต่ำเล็กน้อย ระดับเอนไซม์ตับสูงขึ้นเล็กน้อย โดย aspartate transaminase (AST) เท่ากับ 42 ยูนิตต่อลิตร และ alanine transaminase (ALT) เท่ากับ 47 ยูนิตต่อลิตร ระดับบิลิรูบินและค่าโปรตีนในเลือดอยู่ในเกณฑ์ปกติ ตรวจความสมบูรณ์ของเม็ดเลือด พบเม็ดเลือดขาว (white blood cell; WBC) สูงกว่าปกติเล็กน้อย เท่ากับ 14,260 เซลล์ต่อลูกบาศก์มิลลิเมตร ระดับความเข้มข้นของเลือดและระดับเกล็ดเลือดปกติ ได้รักษาโดยให้สารน้ำ ร่วมกับให้โพแทสเซียมทดแทน และให้ผู้ป่วยนอนพัก ผู้ป่วยดิ้นถีบน้อยลงเป็นลำดับ สองชั่วโมงต่อมา ผู้ป่วยรู้ตัวมากขึ้น ลืมตาได้เอง บ่นปวดศีรษะมาก และจำเหตุการณ์ที่เกิดขึ้นกับตนเองไม่ได้

ตรวจคลื่นไฟฟ้าหัวใจ (electrocardiogram; ECG) แรกรับผลเป็น sinus rhythm with first degree AV block โดยมีอัตราการเต้นของหัวใจ 80 ครั้งต่อนาที ซึ่งประเมินได้ว่า ความผิดปกติแบบ first degree AV block นี้ อาจจะเป็นผลมาจากภาวะขาดออกซิเจน (hypoxia) จึงได้ให้สังเกตอาการไว้ และตรวจติดตามคลื่นไฟฟ้าหัวใจอย่างต่อเนื่อง (ECG monitoring) ตรวจระดับแก๊สในหลอดเลือดแดง (arterial blood gas) ในขณะที่ได้รับออกซิเจนผ่านทางหน้ากากและถุงออกซิเจน (oxygen mask with bag) ที่อัตรา 10 ลิตรต่อนาที ไปแล้วประมาณ 3 ชั่วโมง พบว่ามีระดับออกซิเจนสูง ไม่มีภาวะเลือดเป็นกรด (pH 7.45, pCO_2 44 mmHg, pO_2 331 mmHg, HCO_3 30.2 mEq/L, O_2 saturation 100%)

ในเช้าวันถัดมาผู้ป่วยรู้สึกตัวดี Glasgow coma scale ของเท่ากับ 15 (E = 4, V = 5, M = 6) ตอบคำถามเกี่ยวกับคน สถานที่ และเวลาได้ ยังมีอาการปวดศีรษะมาก ถามเหตุการณ์ในอดีตรู้เรื่อง แต่เหตุการณ์ที่เกิดขึ้นก่อนหมดสติจำไม่ได้ ไม่ทราบว่าตนเองมาอยู่ที่โรงพยาบาลได้อย่างไร วัดสัญญาณชีพปกติ ระดับความอิ่มตัวของออกซิเจนในเลือดเท่ากับ 100 % ได้ทำการส่งตรวจภาพสมองด้วยคลื่นแม่เหล็กไฟฟ้า (Magnetic Resonance Imaging; MRI) ไม่พบความผิดปกติ ไม่มีภาวะสมองบวม และไม่มีเลือดออกในสมอง

ในช่วงเย็นอาการปวดศีรษะลดลง สัญญาณชีพปกติ รับประทานอาหารได้ และไม่มีคลื่นไส้อาเจียน ผู้ป่วยจำเหตุการณ์ได้มากขึ้น สามารถเล่าเหตุการณ์ก่อนหมดสติได้ และจำได้ว่าตนเองเป็นผู้ต่อสายออกซิเจนจากครอบศีรษะของตนเข้ากับวาล์วจ่ายแก๊สผิดชนิด

ในเช้าวันที่ 3 ของการนอนโรงพยาบาล การตรวจติดตามคลื่นไฟฟ้าหัวใจพบว่ามีความผิดปกติแบบ second degree AV block ชนิด Mobitz type I เกิดขึ้นชั่วครู่แล้วหายไป โดยไม่มีอาการเจ็บหน้าอกหรือหมดสติ อายุรแพทย์ระบบหัวใจและหลอดเลือดทำการประเมินอาการแล้ว คาดว่าความผิดปกติที่เกิดขึ้น เป็นจากภาวะขาดออกซิเจนที่ยังเกิดผลกระทบต่อเนื่อง แนะนำให้สังเกตอาการต่อ หลังจากนั้นผู้ป่วยไม่มีความผิดปกติของคลื่นไฟฟ้าหัวใจเกิดขึ้นอีก ในเช้าวันถัดมาผู้ป่วยสามารถกลับ

บ้านได้ การติดตามผู้ป่วยในอีก 1 เดือนถัดมา พบว่าผู้ป่วยหายเป็นปกติ ไม่มีภาวะแทรกซ้อน และสามารถกลับไปทำงานที่โรงงานแห่งเดิมได้อีกครั้งหนึ่ง แต่เปลี่ยนแผนกเป็นแผนกทาสี ซึ่งเป็นแผนกที่ไม่ต้องใส่เครื่องช่วยหายใจแบบครอบศีรษะแทน

วิจารณ์

Simple asphyxiant ออกฤทธิ์ด้วยการแทนที่ออกซิเจนในอากาศ ระดับความเข้มข้นของ simple asphyxiant ที่สูงขึ้น จะทำให้ระดับความเข้มข้นของออกซิเจนในอากาศยิ่งต่ำลง และผลกระทบต่อระบบต่างๆ ในร่างกายจะเพิ่มมากขึ้น อวัยวะในร่างกายที่ได้รับผลกระทบมากที่สุดจากการขาดออกซิเจนได้แก่ ระบบประสาทส่วนกลาง และระบบหัวใจ ตัวอย่างของแก๊สในกลุ่มนี้ ได้แก่ ไนโตรเจน (nitrogen), แก๊สเชื้อเพลิง เช่น มีเทน (methane), อะเซทิลีน (acetylene), โพรเพน (propane), บิวเทน (butane) และแก๊สเฉื่อย เช่น ฮีเลียม และอาร์กอน แก๊สในกลุ่ม simple asphyxiant ทั้งหมดเป็นแก๊สที่ไม่มีสี หลายชนิด เช่น ไนโตรเจน ฮีเลียม และอาร์กอน ไม่มีกลิ่น ทำให้ประสาทสัมผัสมนุษย์รับรู้ถึงการมีอยู่ของแก๊สเหล่านี้ได้ยาก สถานการณ์ที่เสี่ยงต่อการเกิดพิษจาก simple asphyxiant ได้แก่ การอยู่ในที่อับอากาศ (confined space)และสถานการณ์ที่มีการรั่วไหลของแก๊สอัด (compressed gas)[2]

การเกิดภาวะขาดออกซิเจนอันเนื่องจาก simple asphyxiants มีอุบัติการขึ้นเป็นระยะๆ Suruda และ Agnew[3] ได้จำแนกสาเหตุการเสียชีวิตจากภาวะขาดออกซิเจนและการได้รับสารพิษในคนงาน 233 ราย พบว่ามีคนงาน 48 ราย เสียชีวิตจากการได้รับ simple asphyxiants โดย 7 ราย เสียชีวิตจากการได้รับแก๊สอาร์กอน (1 ราย เสียชีวิตจากการต่อท่ออาร์กอนแทนที่ท่ออากาศ) Dorevitch และคณะ[4] ได้จำแนกสาเหตุการเสียชีวิตจากการสูดดมสารพิษของคนงานก่อสร้างในสหรัฐอเมริกาในระยะเวลา 10 ปีพบว่ามีคนงาน 20 รายใน 87 ราย (คิดเป็น 23 %) เสียชีวิตเนื่องจาก simple asphyxiants ในจำนวนนี้ 4 ราย (คิดเป็น 4.6 %) เสียชีวิตจากการได้รับแก๊สอาร์กอน และพบว่าคนที่ทำงานเกี่ยวกับระบบท่อ เช่น ท่อประปา ท่อระบายน้ำ มีความเสี่ยงต่อการเสียชีวิตจากการสูดดมสารพิษสูงกว่าคนงานกลุ่มอื่นเกินกว่า 3 เท่า Hudnall และคณะ[5] ได้รายงานคนงาน 11 ราย เสียชีวิตจากการต่อท่อแก๊สเฉื่อยเชื่อมกับอุปกรณ์ช่วยหายใจในขณะทำงาน ซึ่ง 1 ราย เกิดเหตุจากการต่อเชื่อมกับแก๊สอาร์กอน และ 10 รายเกิดจากการต่อเชื่อมกับแก๊สไนโตรเจน Suruda A และคณะ[6] ได้รายงานสาเหตุของการเสียชีวิตของคนงานที่ใช้อุปกรณ์ช่วยหายใจระหว่างปฏิบัติงาน ในประเทศสหรัฐอเมริกาในระยะเวลา 12 ปี พบว่ามีผู้เสียชีวิตทั้งหมด 45 ราย ซึ่งมีจำนวน 15 ราย ที่เสียชีวิตเนื่องจากความผิดพลาดในการเชื่อมต่อท่อแก๊สเฉื่อยแทนที่ท่ออากาศ NIOSH - Fatality Assessment and Control Evaluation (FACE) Program[7] ได้รายงานการเสียชีวิตของคนงาน 1 ราย ที่ทำงานในท่อขนส่งน้ำมัน ในปี ค.ศ. 1994 สาเหตุการเสียชีวิตของคนงานรายนี้คือการขาดอากาศหายใจ จากการสูดดมแก๊สอาร์กอนหลังจากเข้าไปเปลี่ยนวาล์วออกซิเจนภายในท่อ

Tour และ Aksay[8] ได้รายงานผู้ป่วย 1 ราย ขาดออกซิเจนจากการสูดดมแก๊สไนโตรเจน ผู้ป่วยรายนี้ทำงานพ่นทรายขัดผิวโลหะ ซึ่งได้มีการสวมอุปกรณ์ช่วยหายใจในขณะปฏิบัติงานแต่เกิดความผิดพลาด เนื่องจากเชื่อมต่อท่อไนโตรเจนแทนที่ท่ออากาศ ทำให้เกิดอาการเรียกไม่รู้สึกตัว และหายใจ

เหนื่อย ผู้ป่วยรายนี้ได้รับการรักษาอย่างทันท่วงที และรอดชีวิต

การทำงานในที่อับอากาศหรือใช้เครื่องช่วยหายใจแบบที่มีสายต่ออากาศ (breathing apparatus) นั้น ควรมีผู้มีความรู้คอยควบคุมในระหว่างการทำงานตลอดเวลา ในจากเหตุการณ์ที่เกิดขึ้นแสดงให้เห็นว่า อันตรายจากการใช้อุปกรณ์ช่วยหายใจที่ผิดวิธีอาจทำให้ถึงแก่ชีวิต ผู้ป่วยรายเกิดภาวะขาดอากาศไประยะหนึ่ง แต่มีเพื่อนร่วมงานเข้าไปช่วยเหลืออย่างรวดเร็ว และได้รับการดูแลรักษาอย่างใกล้ชิด จึงสามารถรอดชีวิตมาได้ หากอยู่ในภาวะขาดออกซิเจนนานกว่านี้อาจทำให้เสียชีวิตได้ ระบบความปลอดภัยของโรงงานต่างๆ ที่ใช้อุปกรณ์ช่วยหายใจเหล่านี้ต้องมีการพัฒนาให้ดียิ่งขึ้น การติดสัญลักษณ์ที่ชัดเจน อ่านเข้าใจง่าย แม้จะดูเป็นเรื่องพื้นฐาน แต่ก็เป็นเรื่องที่จำเป็นอย่างยิ่ง การออกแบบรูปร่างหัววาล์วจ่ายแก๊สชนิดต่างๆ ให้มีรูปร่างต่างกัน เพื่อป้องกันไม่ให้เสียบต่อสายแก๊สผิดชนิดได้ (foolproof) และหากพนักงานต่อสายผิด หัววาล์วที่มีรูปร่างต่างกันนี้ก็จะไม่สามารถเสียบกันเข้าน่าจะมีประโยชน์กับโรงงานอย่างยิ่ง

จากเหตุการณ์ในครั้งนี้ ทำให้พิจารณาได้ว่าโรงงานของผู้ป่วยตลอดจนสถานประกอบการทุกแห่ง ที่มีการใช้อุปกรณ์ช่วยหายใจแบบที่ต้องมีการต่อกับสายออกซิเจน ควรดำเนินการเพื่อความปลอดภัยอย่างสูงสุด การทำงานต้องมีเจ้าหน้าที่ความปลอดภัยควบคุม มีการให้ความรู้แก่พนักงานที่ปฏิบัติงาน หัววาล์วจ่ายแก๊สชนิดต่างๆ ควรมีการออกแบบให้มีรูปร่างต่างกัน เพื่อกันไม่ให้เสียบต่อสายแก๊สผิดชนิดได้ (foolproof) ที่วาล์วจ่ายแก๊สและปลายสายต้องมีการติดชื่อแก๊สที่ใช้ไว้อย่างชัดเจน สำหรับพนักงานชาวไทยควรเขียนชื่อแก๊สเป็นภาษาไทย และบริเวณนั้นต้องมีแสงสว่างเพียงพอให้พนักงานสามารถอ่านชื่อแก๊สได้แม้เป็นเวลากลางคืน การทำงานด้วยระบบมีเพื่อนพนักงานคอยเฝ้าดูแล (buddy) ต้องได้รับการสนับสนุนให้ทำทุกครั้ง สิ่งต่างๆ เหล่านี้ จะช่วยลดความเสี่ยงในการเกิดเหตุการณ์เช่นในครั้งนี้ขึ้นอีก และนอกจากนี้ควรอบรมพนักงานให้มีความรู้ในการปฐมพยาบาลเพื่อนพนักงานได้อย่างถูกต้อง และพัฒนาระบบส่งต่อผู้ป่วยจากโรงงานมาที่โรงพยาบาลให้ได้อย่างทันท่วงที เพื่อความปลอดภัยของพนักงานทุกคน

สรุป

ผู้ป่วยรายนี้ได้รับแก๊สอาร์กอน เนื่องจากความผิดพลาดในการต่อสายอากาศเข้าสู่อุปกรณ์ช่วยหายใจแบบครอบศีรษะ (breathing apparatus) ที่ต่อสายออกซิเจนเข้ากับวาล์วจ่ายแก๊สอาร์กอนแทนที่จะต่อเข้ากับวาล์วจ่ายแก๊สออกซิเจน ทำให้ร่างกายได้รับแก๊สอาร์กอนที่มีความเข้มข้นสูงภายในที่ครอบศีรษะ อาร์กอนทำตัวเป็น simple asphyxiant ไปแทนที่ออกซิเจน ทำให้ระดับออกซิเจนในอากาศที่หายใจลดต่ำลง ร่างกายจึงเกิดภาวะขาดออกซิเจนขึ้น และอาการทั้งหมดของผู้ป่วยรายนี้ก็เข้าได้กับสภาวะขาดออกซิเจนอย่างชัดเจน หลังจากได้รับการรักษาด้วยออกซิเจนแล้วคนงานรายนี้สามารถรอดชีวิตได้ในที่สุด

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RISK ASSESSMENT OF FOOD CONTAMINATED WITH RADIOACTIVE ELEMENTS IN THAILAND, NUCLEAR REACTOR ACCIDENT IN JAPAN, 2011

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ABSTRACT

On 11 March 2011, there were earthquake, tsunami and accident at the nuclear power station in Japan, resulting in radioactive contamination in food and environment. Every country including Thailand has monitored and inspected all products originated from Japan. Ministry of Public Health by Thai Food and Drug Administration (Thai FDA) reviewed and cancelled two notifications No. 102 (B.E. 2529) and No. 116 (B.E. 2531) on standards that limit the amount of radioactive contamination in food. According to diasters, Thai FDA issued the new notifications based on four steps of risk assessment: hazard identification, dose-response assessment, exposure assessment and risk characterization. The contents of the new notifications were as followed: the total amount of radioactive elements iodine-131 and cesium-134 and cesium-137, contaminated in food should be less than 100 and 500 Bq/kg, respectively and requirement for imported food products originated from Japan. Both notifications are the major measure of Thai FDA to manage and communicate risk related to food contaminated with radioactive elements.

Keywords: Risk assessment, Iodine-131, Cesium-134, Cesium-137

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การประเมินความเสี่ยงอาหารที่ปนเปื้อนสารกัมมันตรังสีของประเทศไทย กรณีอุบัติเหตุจากโรงไฟฟ้านิวเคลียร์ในประเทศญี่ปุ่น พ.ศ 2554

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บทคัดย่อ

วันที่ 11 มีนาคม พ.ศ. 2554 เกิดแผ่นดินไหว สึนามิ และอุบัติเหตุจากโรงไฟฟ้านิวเคลียร์ในประเทศญี่ปุ่น ทำให้สารกัมมันตรังสีปนเปื้อนในอาหารและสิ่งแวดล้อม ประเทศต่างๆ รวมทั้งประเทศไทยได้มีมาตรการตรวจสอบและเฝ้าระวังอาหารจากประเทศญี่ปุ่น กระทรวงสาธารณสุข โดยสำนักงานคณะกรรมการอาหารและยาได้ทบทวนและยกเลิกประกาศกระทรวงสาธารณสุขฉบับที่ 102 (พ.ศ. 2529) เรื่อง มาตรฐานอาหารที่มีกัมมันตรังสี และประกาศกระทรวงสาธารณสุข ฉบับที่ 116 (พ.ศ. 2531) เรื่อง มาตรฐานอาหารที่มีกัมมันตรังสี (ฉบับที่ 2) และออกประกาศกระทรวงสาธารณสุข ใหม่ 2 ฉบับ คือ ประกาศกระทรวงสาธารณสุข เรื่อง มาตรฐานอาหารที่ปนเปื้อนสารกัมมันตรังสี ลงวันที่ 11 เมษายน 2554 เพื่อกำหนดปริมาณสูงสุดของสารกัมมันตรังสีที่ยอมให้ปนเปื้อนในอาหาร ดังนี้คือ ไอโอดีน-131 ไม่เกิน 100 เบ็กเคอเรลต่อกิโลกรัม (Bq/kg) ซีเซียม-134 และซีเซียม-137 รวมกันไม่เกิน 500 เบ็กเคอเรลต่อกิโลกรัม (Bq/kg) สำหรับเฝ้าระวังอาหารนำเข้าจากประเทศที่มีความเสี่ยงจากอุบัติการณ์การแพร่กระจายของสารกัมมันตรังสี และประกาศกระทรวงสาธารณสุข (ฉบับที่ 341) พ.ศ. 2555 เรื่อง กำหนดเงื่อนไขการนำเข้าอาหารที่มีความเสี่ยงจากการปนเปื้อนสารกัมมันตรังสี ลงวันที่ 5 มีนาคม 2555 เพื่อกำหนดเงื่อนไขและเขตพื้นที่ของประเทศญี่ปุ่นที่ผลิตอาหาร การออกประกาศกระทรวงสาธารณสุขทั้งสองฉบับดำเนินการบนพื้นฐานทางวิทยาศาสตร์ตามหลักการสากล คือ การประเมินความเสี่ยง (Risk assessment) ซึ่งประกอบด้วย 4 ขั้นตอน คือ การบ่งชี้อันตราย การตอบสนองต่อปริมาณ การประเมินการได้รับสัมผัส และการอธิบายความเสี่ยง ประกาศฯ ทั้งสองฉบับจึงเป็นเครื่องมือสำคัญในการบริหารจัดการความเสี่ยงและสื่อสารความเสี่ยงของอาหารที่ปนเปื้อนสารกัมมันตรังสี

คำสำคัญ: การประเมินความเสี่ยง, ไอโอดีน-131, ซีเซียม-134, ซีเซียม-137

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บทนำ

วันที่ 11 มีนาคม 2554 เกิดแผ่นดินไหวที่มีแรงสั่นสะเทือนขนาด 9 ริกเตอร์ และเกิดสึนามิที่ประเทศญี่ปุ่น ห่างจากชายฝั่งเมืองเซนได จังหวัดมิยางิ ประมาณ 80 กิโลเมตร ทำให้ระบบหล่อเย็นแกนปฏิกรณ์ของโรงไฟฟ้านิวเคลียร์ เมืองโอกุมะ จังหวัดฟุกุชิมะ เกาะฮอนชู ไม่ทำงาน เกิดการระเบิดของก๊าซไฮโดรเจนบริเวณภายนอกตัวอาคารเตาปฏิกรณ์ ทำให้สารกัมมันตรังสีแพร่กระจายบริเวณรอบโรงไฟฟ้านิวเคลียร์ และในหลายจังหวัดทางฝั่งตะวันออกของเกาะฮอนชู ได้แก่ จังหวัดฟุกุชิมะ อิบารากิ ชิบะ โตชิกิ และกุนมะ ประเทศญี่ปุ่น ได้ดำเนินมาตรการเกี่ยวกับเหตุการณ์ดังกล่าว ดังนี้ อพยพประชาชนที่อาศัยบริเวณรอบโรงไฟฟ้านิวเคลียร์รัศมี 20 กิโลเมตร และแจกยาโพแทสเซียมไอโอไดด์ให้ประชาชนในพื้นที่กินเพื่อป้องกันมะเร็งต่อมไทรอยด์เนื่องจากการได้รับสารกัมมันตรังสีไอโอดีน-131 พร้อมกับตรวจสอบและเฝ้าระวังการปนเปื้อนสารกัมมันตรังสีในอาหาร น้ำประปา ดิน ฝุ่นละออง และน้ำทะเล ตลอดจนประกาศพื้นที่ซึ่งได้รับผลกระทบจากแผ่นดินไหวและสึนามิ เพื่อจำกัดการบริโภคและห้ามการกระจายอาหารจากบริเวณดังกล่าว[1]

จากเหตุการณ์ดังกล่าว ประเทศต่างๆ ทั่วโลก รวมถึงประเทศไทยได้มีมาตรการตรวจสอบและเฝ้าระวังอาหารจากประเทศญี่ปุ่น ณ ด่านนำเข้าอย่างเข้มงวด และบางประเทศห้ามการนำเข้าอาหารที่มีแหล่งผลิตจากจังหวัดที่ได้รับผลกระทบที่อยู่ในรัศมีของการแพร่กระจายสารกัมมันตรังสี ประเทศไทยโดยสำนักงานคณะกรรมการอาหารและยา กระทรวงสาธารณสุขได้ทบทวนมาตรการทางกฎหมาย คือประกาศกระทรวงสาธารณสุขฉบับที่ 102 (พ.ศ. 2529) เรื่อง มาตรฐานอาหารที่มีกัมมันตรังสี และประกาศกระทรวงสาธารณสุข ฉบับที่ 116 (พ.ศ. 2531) เรื่อง มาตรฐานอาหารที่มีกัมมันตรังสี (ฉบับที่ 2) ซึ่งกำหนดให้อาหารที่ปนเปื้อนฝุ่นกัมมันตรังสีที่ผลิตเพื่อจำหน่าย นำเข้าเพื่อจำหน่าย หรือที่จำหน่าย เป็นอาหารที่กำหนดมาตรฐาน โดยตรวจพบสารกัมมันตรังสีในรูปของซีเซียม-137 (Cs-137) ไม่เกินปริมาณ ดังนี้คือ 1) นมสด 7 เบ็กเคอเรลต่อลิตร (Bq/L) 2) นมผงผลิตภัณฑ์นม และอาหารที่ใช้สำหรับทารก 21 เบ็กเคอเรลต่อกิโลกรัม (Bq/kg) 3) ธัญพืชและอาหารประเภทอื่น 6 เบ็กเคอเรลต่อกิโลกรัม (Bq/kg)[2, 3] ซึ่งค่ากำหนดดังกล่าวพิจารณาบนพื้นฐานจากระดับปริมาณสารกัมมันตรังสีที่มีการตรวจพบในสภาวการณ์ปกติ

การศึกษาครั้งนี้มีวัตถุประสงค์เพื่อประเมินความเสี่ยงจากการบริโภคอาหารที่ปนเปื้อนสารกัมมันตรังสีของประชากรไทยสำหรับเป็นข้อมูลการออกประกาศกระทรวงสาธารณสุข 2 ฉบับคือ ประกาศกระทรวงสาธารณสุข เรื่อง มาตรฐานอาหารที่ปนเปื้อนสารกัมมันตรังสี และประกาศกระทรวงสาธารณสุข ว่าด้วยเรื่อง กำหนดเงื่อนไขการนำเข้าอาหารที่มีความเสี่ยงจากการปนเปื้อนสารกัมมันตรังสี ซึ่งเป็นมาตรการทางกฎหมายที่ใช้เป็นเครื่องมือในการบริหารจัดการและสื่อสารความเสี่ยง เพื่อคุ้มครองความปลอดภัยของผู้บริโภคจากการบริโภคอาหารที่ปนเปื้อนสารกัมมันตรังสีเนื่องจากเหตุฉุกเฉินทางนิวเคลียร์

การประเมินความเสี่ยง (Risk Assessment)

การประเมินความเสี่ยงสารกัมมันตรังสี 3 ชนิดดังกล่าว ประกอบด้วย 4 ขั้นตอนคือ

1. การบ่งชี้อันตราย (Hazard Identification)

อันตรายของสารกัมมันตรังสีทั้งสามชนิด สรุปได้ดังนี้

(1) ไอโอดีน-131 เป็นธาตุที่เกิดจากการผลิตไฟฟ้าจากเตาปฏิกรณ์นิวเคลียร์ที่ใช้ยูเรเนียมเป็นแหล่งพลังงาน และใช้ในทางการแพทย์[4] เพื่อรักษามะเร็งต่อมไทรอยด์ ไทรอยด์เป็นพิษ (Hyperthyroidism) และใช้วินิจฉัยอาการเกี่ยวข้องกับต่อมไทรอยด์[5] ไอโอดีน-131 เป็นธาตุที่มีความคงตัวต่ำเนื่องจากมีค่าครึ่งชีวิตของการสลายตัว (Half-life) สั้นคือ 8 วัน การสลายตัวจะปลดปล่อยรังสีบีตาและรังสีแกมมาออกมา ร่างกายประกอบด้วยไอโอดีนประมาณ 10-20 มิลลิกรัม โดยต่อมไทรอยด์เป็นอวัยวะที่มีไอโอดีนมากที่สุดคือ 90% ของไอโอดีนทั้งหมดที่มีในร่างกาย เมื่อไอโอดีนเข้าสู่ร่างกายโดยการหายใจและ/หรือการกิน จะถูกดูดซึมเข้าสู่กระแสโลหิตได้ทั้งหมดคือ 100% โดยตรวจพบที่ต่อมไทรอยด์ 30% ขับออกทางอุจจาระ 20% และส่วนที่เหลือจะถูกกำจัดออกจากร่างกายในเวลาไม่นานนัก การกำจัดไอโอดีนออกจากร่างกายขึ้นอยู่กับอายุของผู้ที่ได้รับสัมผัส โดยค่าครึ่งชีวิตของการสลายตัวในสิ่งมีชีวิต (Biological half-life) ของทารก เด็กอายุ 5 ปี และผู้ใหญ่ เท่ากับ 11 วัน 23 วันและ 80 วัน ตามลำดับ เนื่องจากต่อมไทรอยด์เป็นอวัยวะที่สะสมไอโอดีนได้มากที่สุด การได้รับสัมผัสไอโอดีนปริมาณมากจึงเพิ่มความเสี่ยงการเกิดมะเร็งต่อมไทรอยด์ ผลการศึกษาทางระบาดวิทยาพบว่า เด็กมีความไวและความเสี่ยงต่อการเกิดมะเร็งต่อมไทรอยด์มากกว่าผู้ใหญ่[4]

(2) ซีเซียม-134 และซีเซียม-137 เป็นธาตุที่เกิดจากการผลิตไฟฟ้าจากเตาปฏิกรณ์นิวเคลียร์ที่ใช้ยูเรเนียมหรือพลูโทเนียมเป็นแหล่งพลังงาน ซีเซียม-134 มีค่าครึ่งชีวิตของการสลายตัว (Half-life) 2 ปีการสลายตัวจะปลดปล่อยรังสีบีตาและแกมมาออกมา ซีเซียม-137 เป็นธาตุที่มีความคงตัวสูงกว่าโดยมีค่าครึ่งชีวิตของการสลายตัว (Half-life) 30 ปี การสลายตัวจะปลดปล่อยรังสีบีตาและแบเรียม-137m (m ย่อมาจาก metastable) ซึ่งมีค่าครึ่งชีวิต 153 วินาที การสลายตัวของแบเรียม-137m จะให้รังสีแกมมา เมื่อซีเซียมเข้าสู่ร่างกายโดยการหายใจและ/หรือการกิน จะถูกดูดซึมเข้าสู่กระแสโลหิตกระจายตัวไปทั่วร่างกาย ถูกกำจัดออกจากร่างกายอย่างรวดเร็ว และอาจสะสมในกล้ามเนื้อ ค่าครึ่งชีวิตของการสลายตัวในสิ่งมีชีวิต (Biological half-life) ในผู้ใหญ่ เท่ากับ 2 วัน อย่างไรก็ตาม การกำจัดซีเซียมออกจากร่างกายในเด็กและวัยรุ่นจะค่อนข้างเร็วเมื่อเทียบกับผู้ใหญ่ การได้รับสัมผัสซีเซียมปริมาณมาก เป็นการเพิ่มความเสี่ยงการเกิดมะเร็ง[6]

2. การตอบสนองต่อปริมาณ (Dose-Response Relationship)

ผลการศึกษาทางระบาดวิทยาเกี่ยวกับการได้รับสารกัมมันตรังสี เช่น การได้รับเรเดียมจากการทำงาน คนงานในเหมืองยูเรเนียม ผู้รอดชีวิตจากระเบิดปรมาณู ผู้ป่วยที่ได้รับการรักษาด้วยสารกัมมันตรังสี พบว่า ผู้ได้รับสารกัมมันตรังสีจะเกิดมะเร็งอย่างมีนัยสำคัญทางสถิติเทียบกับประชากรทั่วไป[7] United state of America, Environmental Protection Agency (US. EPA) คำนวณค่า

สัมประสิทธิ์ความเสี่ยงการเสียชีวิตจากการได้รับสารกัมมันตรังสี (Mortality Risk Coefficients) เนื่องจากการเกิดมะเร็งที่อวัยวะต่างๆ โดยใช้โปรแกรม DCAL (Dose and Risk Calculation) ซึ่งเป็น Age-Dependent Models ที่คำนวณโดยใช้ข้อมูล เช่น เพศ ปริมาณการได้รับสารกัมมันตรังสี เมแทบอลิซึม รายละเอียดตามตารางที่ 1

ค่าสัมประสิทธิ์ความเสี่ยงการเสียชีวิตจากการได้รับสารกัมมันตรังสี (Mortality Risk Coefficients) จากตารางที่ 1 พบว่า ความเสี่ยงจากการเสียชีวิตด้วยมะเร็งจากการได้รับไอโอดีน-131 ซีเซียม-134 และซีเซียม-137 เกิดจากการกินมากกว่าการหายใจ โดยการได้รับซีเซียมจากการกินนั้น 80% มาจากการดื่มน้ำประปา[4, 7]

ตารางที่ 1 แสดงค่าสัมประสิทธิ์ความเสี่ยงการเสียชีวิตจากการได้รับสารกัมมันตรังสี (Mortality Risk Coefficients) 3 ชนิด คือ ไอโอดีน-131 ซีเซียม-134 และซีเซียม-137[4, 7]

Radionuclide	Lifetime Cancer Mortality Risk (pCi^{-1})	
	Inhalation	**Ingestion**
Iodine-131	2.1×10^{-12}	1.4×10^{-11}
Cesium-134	1.1×10^{-11}	3.5×10^{-11}
Cesium-137	8.1×10^{-12}	2.5×10^{-11}

3. การประเมินการได้รับสัมผัส (Exposure Assessment)

มาตรฐานอาหารสากล Codex General Standard for Contaminants and Toxins in Food and Feed (Codex Standard 193-1995) เสนอแนวทางการประเมินการได้รับสัมผัสสารกัมมันตรังสีที่ปนเปื้อนในอาหารกรณีเกิดเหตุฉุกเฉินทางนิวเคลียร์ (Nuclear or Radiological Emergency) โดยคำนวณตามหลักการวิเคราะห์ความเสี่ยง (Risk Analysis) เพื่อให้แต่ละประเทศใช้พิจารณากำหนดค่าความปลอดภัยที่เหมาะสม[8, 9]

3.1 วิธีการคำนวณปริมาณการได้รับสัมผัส

การได้รับสัมผัสสารกัมมันตรังสีจากการบริโภคนั้น ขึ้นอยู่กับปริมาณการบริโภคอาหารที่มีการปนเปื้อน, Ingestion dose coefficients, สัดส่วนของปริมาณการนำเข้าส่งออกของอาหาร ซึ่งคำนวณตามสมการดังนี้

$$E = GL(A) \cdot M(A) \cdot e_{ing}(A) \cdot IPF$$

โดย

E = the mean internal dose of the public หมายถึง ปริมาณการได้รับสัมผัสหรือ ปริมาณการได้รับสารกัมมันตรังสี (A) จากการบริโภคอาหารนำเข้าที่มีการปนเปื้อนของประชากรที่ระดับเฉลี่ย หน่วยเป็นมิลลิซีเวิร์ต

GL(A) = Guideline levels หมายถึง ค่าความปลอดภัยหรือค่ากำหนดปริมาณสารกัมมันตรังสีที่ปนเปื้อนในอาหารนำเข้าที่ประชากรทั่วไปบริโภคโดยไม่เกิดอันตรายต่อสุขภาพ หน่วยเป็นเบ็กเคอเรลต่อกิโลกรัมอาหาร

M(A) = the age-dependent mass of food consumed per year หมายถึง ปริมาณการบริโภคอาหารต่อปี หน่วยเป็นกิโลกรัมต่อคนต่อปี

$e_{ing}(A)$ = the age-dependent ingestion dose coefficient หมายถึง ค่าสัมประสิทธิ์ของผลกระทบจากการบริโภคอาหารที่ปนเปื้อนสารกัมมันตรังสีต่อช่วงอายุมนุษย์ หน่วยเป็นมิลลิซีเวิร์ตต่อเบ็กเคอเรล

IPF = the import/production factor หมายถึง สัดส่วนของปริมาณอาหารที่นำเข้าจากพื้นที่ที่ปนเปื้อนสารกัมมันตรังสีต่อปริมาณอาหารที่ผลิตและนำเข้าทั้งหมดในภูมิภาคหรือประเทศที่พิจารณา

3.2 ข้อมูลที่ใช้คำนวณปริมาณการได้รับสัมผัส

GL(A) พิจารณาโดยใช้ค่ากำหนดการปนเปื้อนสารกัมมันตรังสี ไอโอดีน-131 ซีเซียม-134 และซีเซียม-137 ในอาหารตามมาตรฐานโคเด็กซ์ รายละเอียดตามตารางที่ 2

M(A) พิจารณาโดยใช้ข้อมูลการบริโภคอาหารของคนไทย จากประชากร 2 กลุ่มอายุ คือ 1) กลุ่มทารก (infant) อายุ 0-1 ปี ซึ่งใช้ข้อมูลการบริโภคของกลุ่มอายุ 0-3 ปี และ 2) กลุ่มประชากรทั่วไป (adult) อายุมากกว่า 3 ปีขึ้นไป รายละเอียดตามตารางที่ 3

e_{ing}(A) เป็นค่าซึ่งทบวงการพลังงานปรมาณูระหว่างประเทศ (International Atomic Energy Agency: IAEA) ได้จัดทำข้อมูลของค่าสัมประสิทธิ์ของผลกระทบจากการหายใจและการบริโภคอาหารที่ปนเปื้อนสารกัมมันตรังสีต่อช่วงอายุมนุษย์ โดยกลุ่มทารกจะมีความเสี่ยงมากกว่ากลุ่มผู้ใหญ่ เพื่อเป็นข้อมูลสำหรับนำมาใช้ประเมินความเสี่ยงจากการได้รับสัมผัสสารกัมมันตรังสีต่างๆ ทั้งจากธรรมชาติและมนุษย์สร้างขึ้น รายละเอียดตามตารางที่ 4

IPF พิจารณาโดยใช้การอนุมานว่าหลังจากเกิดเหตุฉุกเฉินทางนิวเคลียร์ สารกัมมันตรังสีปนเปื้อนสู่สิ่งแวดล้อมและไม่สามารถหาแหล่งอาหารทดแทนอาหารที่ปนเปื้อนสารกัมมันตรังสีได้ องค์การอาหารและเกษตรแห่งสหประชาชาติ (Food and Agriculture Organization; FAO) ได้รวบรวมและสรุปผลการศึกษาทางสถิติเกี่ยวกับการนำเข้าอาหารทั่วโลกในสถานการณ์ปกติ ได้ค่า IPF = 0.1 [8,9]

<u>ตัวอย่างการคำนวณ</u> กลุ่มทารกได้รับสัมผัสไอโอดีน-131

$E = 100\ Bq/kg \cdot 267.46\ kg \cdot 1.8 \cdot 10^{-4}\ mSv/Bq \cdot 0.1$

$= 0.48\ mSv$

ตารางที่ 2 ค่ากำหนดการปนเปื้อนสารกัมมันตรังสีในอาหารตามมาตรฐานโคเด็กซ์[9]

ชนิดของสารกัมมันตรังสี	ค่ากำหนดการปนเปื้อนของสารกัมมันตรังสี (เบ็กเคอเรลต่อกิโลกรัมอาหาร; Bq/kg)	
	อาหารทารก	อาหารอื่น
ไอโอดีน-131 (^{131}I, Iodine-131)	100	100
ซีเซียม-134 (^{134}Cs, Cesium-134)	1,000	1,000
ซีเซียม-137 (^{137}Cs, Cesium-137)	1,000	1,000

ตารางที่ 3 ข้อมูลการบริโภคอาหารของคนไทย[10]

กลุ่มประชากร	ประเภทอาหาร	ลักษณะของข้อมูลการบริโภคที่ใช้ในการคำนวณ	ปริมาณการบริโภคอาหาร (กิโลกรัม/คน/ปี)	
			เฉลี่ย	97.5 เปอร์เซ็นต์ไทล์
กลุ่มทารก อายุ 0-1 ปี	รายการอาหารที่ทารกมีการบริโภค (นมผงสำหรับทารก อาหารสำเร็จรูปสำหรับทารก และอาหารเสริมสำหรับทารก)	Eater only ของกลุ่มอายุ 0-3 ปี	267.46	506.38
	High Scenario กรณีที่มีการบริโภคนมผงสำหรับทารกในปริมาณสูง	Eater only ของกลุ่มอายุ 0-3 ปี ยกเว้นรายการนมผงสำหรับทารกที่ใช้ข้อมูลที่ 97.5 เปอร์เซ็นต์ไทล์	**356.11**	-
กลุ่มประชากรทั่วไป อายุ > 3 ปี ขึ้นไป	ทุกรายการอาหาร	Per capita ของประชากรทั่วไป	667.49	4091.17
	High Scenario กรณีที่มีการบริโภคข้าวเจ้าในปริมาณสูง	Per capita ของประชากรทั่วไป ยกเว้นรายการข้าวเจ้า ที่ใช้ข้อมูลที่ 97.5 เปอร์เซ็นต์ไทล์	**929.34**	-

ตารางที่ 4 แสดงค่า Age-dependent ingestion dose coefficient (dose per unit intake, mSv/Bq)[8]

Radionuclide	Age-dependent ingestion dose coefficient (mSv/Bq)	
	infant	adult
Iodine-131 (^{131}I)	1.8×10^{-4}	2.2×10^{-5}
Cesium-134 (^{134}Cs)	2.6×10^{-5}	1.9×10^{-5}
Cesium-137 (^{137}Cs)	2.1×10^{-5}	1.3×10^{-5}

3.3 ผลการประเมินการได้รับสัมผัสสารกัมมันตรังสีจากการบริโภคอาหารของประชากรไทย รายละเอียดตามตารางที่ 5

พิจารณาจากตารางที่ 5 พบว่า

กลุ่มทารก อายุ 0-1 ปี ได้รับสัมผัสซีเซียม-134 มากที่สุด เท่ากับ 0.7 mSv/ปี ที่ระดับเฉลี่ย และ 0.93 mSv/ปี ที่ High Scenario รองลงมาเป็นซีเซียม-137 และไอโอดีน-131 ตามลำดับ

กลุ่มประชากรทั่วไป ได้รับสัมผัสซีเซียม-134 มากที่สุด เท่ากับ 1.27 mSv/ปี ที่ระดับเฉลี่ย และ 1.77 mSv/ปี ที่ High Scenario รองลงมาเป็นซีเซียม-137 และไอโอดีน-131 ตามลำดับ

กลุ่มทารกได้รับสัมผัสไอโอดีน-131 สูงกว่ากลุ่มประชากรทั่วไป ทั้งระดับเฉลี่ยและ High Scenario ขณะที่การได้รับสัมผัสซีเซียม-134 และซีเซียม-137 ของกลุ่มประชากรทั่วไปสูงกว่ากลุ่มทารก ทั้งระดับเฉลี่ยและ High Scenario

ตารางที่ 5 ผลการประเมินการได้รับสัมผัสสารกัมมันตรังสีจากการบริโภคอาหารของประชากรไทย

กลุ่มประชากร		ปริมาณสารกัมมันตรังสีที่ได้จากการบริโภคอาหารต่อปี (mSv/ปี)		
		ไอโอดีน-131	ซีเซียม-134	ซีเซียม-137
ทารก อายุ 0-1 ปี	ระดับเฉลี่ย	0.48	0.7	0.56
	High Scenario	0.64	0.93	0.75
ประชากรทั่วไป อายุ > 3 ปี ขึ้นไป	ระดับเฉลี่ย	0.15	1.27	0.87
	High Scenario	0.2	1.77	1.21

4. การอธิบายความเสี่ยง (Risk Characterization)

เมื่อเปรียบเทียบปริมาณสารกัมมันตรังสีที่ปนเปื้อนจากอาหารนำเข้าที่ประชากรไทยบริโภค (the mean internal dose of the public: E) (ตารางที่ 5) กับ Intervention Exemption Level of Dose หรือปริมาณการได้รับสัมผัสสารกัมมันตรังสีหลังเกิดเหตุฉุกเฉินทางนิวเคลียร์หนึ่งปี โดยไม่เกิดอันตรายต่อสุขภาพ เท่ากับ 1 mSv per year ดังนี้คือ

4.1 ปริมาณไอโอดีน-131 ที่ปนเปื้อนจากอาหารนำเข้าที่ทารกและผู้ใหญ่บริโภคเป็นระยะเวลาหนึ่งปี กรณีบริโภคอาหารปริมาณเฉลี่ยและ/หรือ High Scenario ไม่เกิน 1 mSV จัดว่าปลอดภัย

4.2 ปริมาณซีเซียม-134 และปริมาณซีเซียม-137 ที่ปนเปื้อนจากอาหารนำเข้าที่ทารกบริโภคเป็นระยะเวลาหนึ่งปี กรณีบริโภคอาหารปริมาณเฉลี่ยและ/หรือ High Scenario ไม่เกิน 1 mSV จัดว่าปลอดภัย

4.3 ปริมาณซีเซียม-137 ที่ปนเปื้อนจากอาหารนำเข้าที่ผู้ใหญ่บริโภคเป็นระยะเวลาหนึ่งปี กรณีบริโภคอาหารปริมาณเฉลี่ย ไม่เกิน 1 mSV จัดว่าปลอดภัย

4.4 ปริมาณซีเซียม-134 ที่ปนเปื้อนจากอาหารนำเข้าที่ผู้ใหญ่บริโภคเป็นระยะเวลาหนึ่งปี กรณีบริโภคอาหารปริมาณเฉลี่ยและ/หรือ High Scenario เกิน 1 mSV จัดว่าไม่ปลอดภัย

4.5 ปริมาณซีเซียม-137 ที่ปนเปื้อนจากอาหารนำเข้าที่ผู้ใหญ่บริโภคเป็นระยะเวลาหนึ่งปี กรณีบริโภคอาหารปริมาณ High Scenario เกิน 1 mSV จัดว่าไม่ปลอดภัย

ข้อสังเกตผลการประเมินการได้รับสัมผัสสารกัมมันตรังสีจากการบริโภคอาหารของประชากรไทย

1.ผลการประเมินความเสี่ยงดังกล่าวมีข้อสังเกต ดังนี้

1.1 ข้อมูลการบริโภคอาหารของกลุ่มทารกอายุ 0-3 ปี ไม่ได้แยกเป็นกลุ่มย่อยคือทารก 0-1 ปี ดังนั้น ข้อมูลที่นำมาคำนวณอาจไม่เฉพาะเจาะจงและประเมินมากกว่าความเป็นจริง (over-estimate) เทียบกับข้อมูลของโคเด็กซ์ ซึ่งใช้ข้อมูลการบริโภคอาหารของทารก 0-1 ปี

1.2 The import/production factor ซึ่งใช้ในการคำนวณเท่ากับ 0.1 โดยอ้างอิงจากการคำนวณค่า Guideline levels ของโคเด็กซ์ ทั้งทารกและประชากรทั่วไป โดยในกรณีของทารกปริมาณอาหารนำเข้าที่ปนเปื้อนสารกัมมันตรังสีสำหรับทารก อาจไม่เท่ากับ 0.1 เนื่องจากทารกบริโภคน้ำนมเป็นอาหารหลัก บริโภคอาหารอื่นเป็นส่วนน้อย และประเทศไทยไม่ได้นำเข้าน้ำนมสำหรับทารกจากประเทศญี่ปุ่นเป็นหลัก

1.3 สารกัมมันตรังสีตรวจพบได้ทุกหนทุกแห่งในธรรมชาติ และอาจตรวจพบในอาหารปริมาณแตกต่างกันได้ถึงร้อยเท่า ซึ่งเป็นปัจจัยที่ไม่สามารถควบคุมได้ ทั้งนี้การประเมินความเสี่ยงสำหรับกรณีนี้เป็นการประเมินความเสี่ยงการได้รับอันตรายจากอาหารที่ปนเปื้อนสารกัมมันตรังสีจากเหตุฉุกเฉินทางนิวเคลียร์เท่านั้น ไม่ได้รวมปริมาณสารกัมมันตรังสีในธรรมชาติ

1.4 การเฝ้าระวังอาหารนำเข้าอย่างเข้มงวดของประเทศต่างๆ การกำกับดูแลปัญหาของประเทศญี่ปุ่นหลังจากเกิดอุบัติเหตุจากโรงไฟฟ้านิวเคลียร์ มาตรการต่างๆ ทางการเกษตร การเปลี่ยนย้ายแหล่งที่มาหรือพื้นที่ที่ผลิตของอาหาร และระยะเวลาของการสลายตัวของสารกัมมันตรังสี ทำให้อัตราส่วนของอาหารปนเปื้อนสารกัมมันตรังสีในประเทศต่างๆ ทั่วโลกลดลง มีการศึกษาพบว่าอัตราส่วนอาหารนำเข้าที่ปนเปื้อนสารกัมมันตรังสีหลังเกิดเหตุฉุกเฉินทางนิวเคลียร์หนึ่งปี ลดลงได้สูงสุดถึงหนึ่งร้อยเท่า อย่างไรก็ตาม อาหารบางชนิด เช่น ผลิตภัณฑ์จากป่ายังคงตรวจพบการปนเปื้อนสารกัมมันตรังสีและการปนเปื้อนดังกล่าวเพิ่มมากขึ้น ดังนั้นปริมาณสารกัมมันตรังสีของแต่ละคน ซึ่งเกิดจากการบริโภคอาหารปนเปื้อนสารกัมมันตรังสีจะลดลงจนถึงปริมาณที่ไม่มีผลต่อสุขภาพ ต้องใช้ระยะเวลานานหลายปี

2. ข้อมูลที่นำมาใช้ในการประเมินความเสี่ยงได้แก่ ข้อมูลการเปรียบเทียบปริมาณสารกัมมันตรังสีที่ปนเปื้อนจากอาหารนำเข้าที่ประชากรไทยบริโภค ซึ่งใช้ข้อมูลการบริโภคอาหารของประชากรไทย และค่า Intervention Exemption Level of Dose 1 mSv/ปี ซึ่งเป็นปริมาณการได้รับสัมผัสสารกัมมันตรังสีหลังเกิดเหตุฉุกเฉินทางนิวเคลียร์หนึ่งปี โดยที่ไม่เกิดอันตรายต่อสุขภาพ พบว่า ปริมาณซีเซียม-134 ที่ปนเปื้อนจากอาหารนำเข้าที่ระดับเฉลี่ย และ High Scenario ของประชากรทั่วไป และปริมาณซีเซียม-137 ที่ปนเปื้อนจากอาหารนำเข้าที่ High Scenario ของ

ประชากรทั่วไป มีค่าเกิน 1 mSv/ปี ซึ่งจัดว่าไม่ปลอดภัย ได้เสนอเป็นข้อสังเกตของการประเมินความเสี่ยงครั้งนี้ให้คณะกรรมการอาหารและคณะอนุกรรมการฯ พิจารณาการกำหนดมาตรฐานอาหารที่ปนเปื้อนสารกัมมันตรังสีของประเทศไทย

แนวทางการกำหนดมาตรฐานอาหารที่ปนเปื้อนสารกัมมันตรังสี

ประกาศกระทรวงสาธารณสุข ฉบับที่ 102 (พ.ศ.2529) เรื่อง มาตรฐานอาหารที่มีกัมมันตรังสี และประกาศกระทรวงสาธารณสุข ฉบับที่ 116 (พ.ศ. 2531) เรื่อง มาตรฐานอาหารที่มีกัมมันตรังสี (ฉบับที่ 2)[2, 3] พบว่าการกำหนดค่ามาตรฐานการปนเปื้อนสารกัมมันตรังสีดังกล่าวเป็นการพิจารณาบนพื้นฐานจากระดับปริมาณสารกัมมันตรังสีที่มีการตรวจพบในสภาวการณ์ปกติ ซึ่งค่ากำหนดดังกล่าวไม่สอดคล้องกับแนวทางของมาตรฐานสากล สำนักงานคณะกรรมการอาหารและยา ได้ตระหนักถึงความสำคัญของกระบวนการประเมินความเสี่ยงจากการปนเปื้อนสารกัมมันตรังสีในอาหาร จึงพิจารณาผลการประเมินความเสี่ยงจากการได้รับสัมผัสสารกัมมันตรังสีจากการบริโภคอาหารของประชากรไทย รวมถึงปัจจัยต่างๆ ที่เกี่ยวข้อง ได้แก่ ระยะเวลาในการสลายตัวครึ่งชีวิต (Half-life) ของสารกัมมันตรังสี สถานการณ์และโอกาสของการปนเปื้อน ระยะเวลา ข้อจำกัดของการตรวจวิเคราะห์ทางห้องปฏิบัติการ ข้อกำหนดชนิดสารกัมมันตรังสี และค่ามาตรฐานการปนเปื้อนสารกัมมันตรังสีของประเทศต่างๆ แล้ว เห็นควรให้กำหนดค่ามาตรฐานการปนเปื้อนสารกัมมันตรังสี 3 ชนิด ได้แก่ สารกัมมันตรังสี ไอโอดีน-131 ซีเซียม-134 และซีเซียม-137 ซึ่งสอดคล้องกับแนวทางของหลายประเทศที่มีการตรวจสอบเฝ้าระวังอาหารนำเข้าในขณะนั้น โดยกำหนดค่ามาตรฐานปริมาณการปนเปื้อนสารกัมมันตรังสีในอาหาร ดังนี้

(1) ไอโอดีน-131 ไม่เกิน 100 Bq/kg อ้างอิงตาม Guideline Levels ของโคเด็กซ์ ซึ่งผลการประเมินการได้รับสัมผัสไม่เกิน 1 mSV (ตามตารางที่ 5)

(2) ซีเซียม-134 และซีเซียม-137 รวมกันไม่เกิน 500 Bq/kg โดยกำหนดต่ำกว่า Guideline Levels ของโคเด็กซ์ เนื่องจากผลการประเมินการได้รับสัมผัสซีเซียม-134 และซีเซียม-137 อ้างอิงตาม Guideline Levels ของโคเด็กซ์ (ตามตารางที่ 2) ของประชากรไทยต่อปี มีค่าเกิน 1 mSV (ตามตารางที่ 5) ในกลุ่มประชากรทั่วไป ซึ่งกำหนดให้สอดคล้องกับค่ากำหนดซีเซียม-134 และซีเซียม-137 สำหรับอาหารทั่วไป (ยกเว้น นม ผลิตภัณฑ์นม และน้ำบริโภค) ของประเทศญี่ปุ่น [11, 12]

การกำหนดมาตรการทางกฎหมายเพื่อคุ้มครองผู้บริโภคในประเทศ

จากข้อมูลที่ได้นำเสนอดังกล่าวข้างต้น สำนักงานคณะกรรมการอาหารและยาเห็นควรให้ออกประกาศกระทรวงสาธารณสุขเพื่อเป็นมาตรการทางกฎหมายสำหรับควบคุมอาหารที่มีความเสี่ยงจากการปนเปื้อนสารกัมมันตรังสี เป็น 2 ฉบับคือ

1. ประกาศกระทรวงสาธารณสุข เรื่อง มาตรฐานอาหารที่ปนเปื้อนสารกัมมันตรังสี[13] เพื่อกำหนดปริมาณสารกัมมันตรังสีที่ปนเปื้อนในอาหาร สำหรับเฝ้าระวังอาหารนำเข้า ดังนี้คือ ไอโอดีน-131 ไม่เกิน 100 Bq/kg ซีเซียม-134 และ

ซีเซียม-137 รวมกันไม่เกิน 500 Bq/kg โดยปริมาณไอโอดีน-131 อ้างอิงตาม Guideline Levels ของโคเด็กซ์ สำหรับซีเซียม-134 และซีเซียม-137 เนื่องจากผลการประเมินการได้รับสัมผัสพบว่าประชากรทั่วไปยังคงมีความเสี่ยงจากการได้รับสารกัมมันตรังสี ซีเซียม-134 และซีเซียม-137 จากการบริโภคอาหาร ดังนั้นจึงกำหนดให้ค่าของซีเซียม-134 และซีเซียม-137 ต่ำกว่า Guideline Levels ของโคเด็กซ์ ซึ่งกำหนดปริมาณของซีเซียม-134 และซีเซียม-137 ไว้ไม่เกิน 1,000 Bq/kg

ทั้งนี้สำนักงานคณะกรรมการอาหารและยา ได้พิจารณาตามแนวทาง Multiple radionuclides in foods ของโคเด็กซ์ ที่มีข้อแนะนำ Guideline Levels ของสารกัมมันตรังสี ในอาหาร 2 กลุ่ม คือ อาหารทารก และอาหารอื่น โดยสารกัมมันตรังสีที่อยู่ในกลุ่มเดียวกันนั้น อาจกำหนดแยกตามแต่ละชนิดของสารกัมมันตรังสี หรืออาจกำหนดรวมไว้ด้วยกัน ประกอบกับข้อมูลการเฝ้าระวังของประเทศญี่ปุ่น ยังพบว่ามีการปนเปื้อนซีเซียมทั้ง 2 ไอโซปโทป เกินค่า Guideline Levels ของโคเด็กซ์ ดังนั้นเพื่อคุ้มครองผู้บริโภคให้ได้รับสัมผัสสารกัมมันตรังสีดังกล่าวน้อยที่สุด จึงกำหนดค่ามาตรฐานต่ำกว่า Guideline Levels ของโคเด็กซ์ คือ ซีเซียม-134 และซีเซียม-137 รวมกัน ไม่เกิน 500 Bq/kg อ้างอิงตามค่ากำหนดการปนเปื้อนสารกัมมันตรังสีสำหรับอาหารทั่วไป (ยกเว้น นมผลิตภัณฑ์นม และน้ำบริโภค) ของประเทศญี่ปุ่น[12,14]

2. ประกาศกระทรวงสาธารณสุข (ฉบับที่ 341) พ.ศ. 2555 เรื่อง กำหนดเงื่อนไขการนำเข้าอาหารที่มีความเสี่ยงจากการปนเปื้อนสารกัมมันตรังสี[15] เพื่อกำหนดเขตพื้นที่ของประเทศญี่ปุ่นที่ผลิตอาหารซึ่งอยู่ในรัศมีที่มีการรั่วไหลของสารกัมมันตรังสีจากโรงไฟฟ้านิวเคลียร์และกำหนดเงื่อนไขที่ต้องปฏิบัติให้เป็นไปตามเรื่อง มาตรฐานอาหารที่ปนเปื้อนสารกัมมันตรังสี [16,17,18]

ประกาศกระทรวงสาธารณสุขทั้งสองฉบับ เป็นเครื่องมือสำคัญในการบริหารความเสี่ยง (Risk Management) และสื่อสารความเสี่ยง (Risk Communication) ของการได้รับสารกัมมันตรังสีปนเปื้อนอาหารนำเข้าจากประเทศญี่ปุ่นของประเทศไทย ซึ่งสำนักงานคณะกรรมการอาหารและยาได้ดำเนินการบนพื้นฐานทางวิทยาศาสตร์ตามหลักการประเมินความเสี่ยง (Risk Assessment) โดยมีวัตถุประสงค์เพื่อกำกับดูแลอาหารนำเข้าจากประเทศญี่ปุ่น ซึ่งอาจปนเปื้อนสารกัมมันตรังสี

กิตติกรรมประกาศ

ขอขอบคุณคณะกรรมการอาหารและคณะอนุกรรมการพิจารณากำหนดคุณภาพมาตรฐานและหลักเกณฑ์วิธีการและเงื่อนไขในการควบคุมอาหาร สำหรับการพิจารณาข้อมูล เพื่อออกประกาศกระทรวงสาธารณสุข 2 ฉบับคือ ประกาศกระทรวงสาธารณสุข เรื่อง มาตรฐานอาหารที่ปนเปื้อนสารกัมมันตรังสี และประกาศกระทรวงสาธารณสุข ว่าด้วยเรื่อง กำหนดเงื่อนไขการนำเข้าอาหารที่มีความเสี่ยงจากการปนเปื้อนสารกัมมันตรังสี และขอขอบคุณสำนักงานปรมาณูเพื่อสันติ สำหรับการให้ความรู้ความเข้าใจเกี่ยวกับสารกัมมันตรังสีและนิวเคลียร์ รวมถึงให้ความร่วมมือในการตรวจวิเคราะห์เพื่อเป็นข้อมูลในการเฝ้าระวังการปนเปื้อนสารกัมมันตรังสีในอาหาร

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